SER. II, VOL. 21 *April 1959* NO. 6

TRANSACTIONS
OF
THE NEW YORK ACADEMY OF SCIENCES

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Transactions of The New York Academy of Sciences, Series II, Volume 21, No. 6
April 1959

Second-class postage paid at New York, N. Y. This publication is distributed to members and is published monthly from November to June, inclusive, by The New York Academy of Sciences, 2 East Sixty-third Street, New York 21, N. Y.

Executive Director: EUNICE THOMAS MINER

SECTION OF GEOLOGICAL SCIENCES

ENGINEERING GEOLOGY OF THE CITY OF NEW YORK WATER SUPPLY SYSTEM*

Thomas W. Fluhr

Geology and Soils Division, Board of Water Supply of the City of New York, Downsville, N. Y.

I propose to discuss the geologic work carried on by the Board of Water Supply of the City of New York. After completion of the Catskill water supply system, the board in 1937 began construction of the Delaware aqueduct system. That system now includes the 85-mile-long Delaware Aqueduct, the 25½-mile-long East Delaware Aqueduct, and the Merriman, Neversink, and Downsville dams and their associated reservoirs.[1] We are now proceeding with construction of the 44-mile-long West Delaware Tunnel and have begun construction of the Cannonsville Dam and its reservoir. It will be of interest to see how engineering geology has played a part in the planning and construction of these works.

The geologic work of the Board of Water Supply is carried on by its Geology and Soils Division. The work of that division includes: preliminary planning for future work, investigations for work proposed for construction in the near future, control and advisory services for works under construction, and making records of the results of work as construction is carried on. The scope of the work includes: (1) investigations of dam sites, tunnels, shaft sites, and intake and outlet works; (2) investigations for bridge foundations; (3) geologic work in connection with highways; (4) assistance to the Bureau of Claims in acquisition of properties; (5) assistance to the Law Department and expert testimony in connection with claims.

Methods of Subsurface Investigation

The Board of Water Supply makes most of its subsurface investigations by means of test pits and test borings. Unfortunately, geophysical methods do not yield results sufficiently precise for most engineering work. Test borings for the board are made under contract by drilling companies that specialize in such work. Soil samples are obtained by means of split-spoon samplers, thin-walled steel tubing or, at times, by drilling soil cores. Rock cores are recovered by diamond drilling, using double-tube swivel-type core barrels.

Investigations for Tunnels[2]

When planning a tunnel many factors, not all geologic, must be con-

*This paper, illustrated with slides, was presented at a meeting of the Section on March 2, 1959.

sidered in order to minimize construction costs. The major objectives are to keep the tunnel as short as possible, to select locations for intermediate shafts where the shafts will be as shallow as possible, and to locate shaft and tunnel sites at places where the access is easy and the land is undeveloped. Borings are made at tunnel portals, shaft sites, and at various intermediate points.

One geologic problem associated with the construction of tunnel portals is that of stress relief in the rock. Erosion has unloaded the rock in valleys more than it has on hills. The imbalance causes stress in the rock. Relief of that stress produces steeply dipping joints that trend parallel to the direction of the valleys. In consequence, many tunnel portals and adjacent tunnels penetrate badly jointed rock.

Highway Construction

Construction of a dam creates a reservoir, flooding a valley and entirely or partially submerging the existing roads. When the city of New York builds a reservoir it must construct highways to replace those submerged. This is an undertaking of considerable extent; for example, about fifty-three miles of substituted highways were required around the Pepacton Reservoir alone. At the present time the city acquires the land and designs the highways, which are then constructed by the New York State Department of Public Works, but the construction costs are paid by the city of New York. The design of the highways is quite complicated.

After preliminary design of a highway has been made, the geologist walks over the ground, notes the location of rock outcrops, and estimates the depth to bedrock where outcrops are lacking. With this data at hand, a final design is prepared. In critical locations supplementary data are obtained by means of seismic work or test borings.

Bridge Foundations

Where substituted highways cross reservoirs, bridges must be constructed. Some of these are large and costly structures. For example, the Shavertown Bridge crossing the Pepacton reservoir is some one thousand feet in length and cost more than two million dollars.[3] The locations of these bridges are fixed by highway alignment. A preliminary design is made and pier and abutment sites are tested by means of borings. Soil samples and rock cores are examined and classified. A geologic section is drawn up showing the various rock and soil foundations. With this help, design for the bridge foundation can be made. Types of foundations used include open excavations to bedrock, steel H columns to bedrock, and concrete piles.

Tunneling Through Faults

Most rock tunnels at some place or other pass through weak zones where the rock may be jointed, crushed, sheared, decayed, or water-bearing. In some cases the weakness in the rock comes from actual rock movement; in other cases it appears to be simple decay extending to depth. Such weak zones, whatever their origin, are usually described as "fault zones."

The main Delaware Aqueduct passes through many such zones.[4] In the Catskill region these zones are less frequent. They vary in intensity from simple jointed zones to zones where the rock is granulated or crushed and badly decayed. Usually water follows such zones and is the principal cause of trouble. In addition to the nuisance of incoming water, it may carry in crushed rock and fault gouge. Much of the early literature in engineering geology deals with fault and decayed zones. With years of experience in handling them their problems do not appear as formidable as formerly.

The presence of some faults is unpredictable. This appears to be especially true in the Catskills, where faults sometimes are found under ridges, with no topographic expression of their presence. However, in most places faults follow formational contacts or underlie valleys or swamps. Where even slight evidence of faulting or decay is apparent, it is wise to make subsurface exploration by means of borings. The use of angle borings often facilitates detailed exploration of faults so that their width and characteristics at tunnel grade can be evaluated. This was done at several places on the chief Delaware Aqueduct line.

Penetration of fault and decayed zones depends on three primary procedures, which of course may be employed in various combinations. The most important thing to do is to control inflowing water. This may be done in two ways: (1) the use of grouting to seal up crevices and fissures in the rock; and (2) the use of drainage. Drill holes or small tunnel drifts may prove useful to drain off the ground water. Once the ground water has been drained away, the material can be penetrated by soft-ground tunneling methods. A third method in tunneling through fault zones is to use support to hold the weakened rock in place. There are innumerable variations in all these methods. The method or combination of methods to be employed depends to some degree on geologic factors. It also depends on nongeological factors, such as the contractor's price for grouting as compared with his price for pumping, on the availability and costs of equipment, and on the experience of his staff. Once a method has been pursued by a contractor it is difficult, if not impossible, to decide whether he has adopted the best and most economical course. There is no general rule for attacking the problem of fault zones. Each is an individual problem that must be decided on its merits.

The fact remains that fault and decayed zones may add greatly to the

cost of a tunneling project. The engineer is therefore justified in spending a considerable amount of effort and money in locating and exploring them.

Support in Rock Tunnels

Years ago tunnels in rock were driven with little or no support. Even today in some parts of the country little attention is paid to tunneling support. Years ago, of course, many tunnel workers were killed by rock falls, so that tunneling has gained a reputation as a hazardous occupation. Today our tunnels are much safer than they were; rock falls are infrequent.

In a few places rock is of such good quality that tunnels may be driven without support. Such places are rare. Most geologists have some acquaintance with popping rock. Hard, massive, uniform rock masses may be elastically deformed. When tunnels penetrate such rock it snaps and pops off in small or large slabs, and is recognized as dangerous. What many geologists do not realize is that many rock formations are stressed, but that the relief of stress does not take place instantaneously, as with popping rock. Instead, the stress relief occurs gradually. Such rock often appears hard and sound when excavation is first made into it. After the lapse of about a week the tunnel roof begins to crack and spall and, unless supported, rock falls may occur. Formerly it was customary to have safety miners test the rock in tunnels and scale down loose pieces. Today tunnel driving is organized and mechanized so that rapid progress is attained. There is no time for scaling loose rock. It has proved more satisfactory from the standpoints of both safety and progress to install continuous roof support.

Formerly tunnel supports consisted of steel or timber bents that were costly and required time and labor for installation. During the excavation of the East Delaware Tunnel about eight years ago, the use of rock bolting was introduced. Rock bolting had been used in some mines for many years, but was first used extensively in the eastern part of the country on the East Delaware Aqueduct. It can be installed rapidly, does not delay progress, and has proved successful in supporting tunnel roofs. It has even proved successful in supporting the crushed and decayed rock of fault zones. It is only when a tunnel penetrates an extremely poor quality of rock for a long distance that steel bents must be used. On the West Delaware Tunnel rock bolting has almost entirely displaced steel or timber bents for tunnel support. Although some geologists still debate means of judging whether a tunnel will require support and the amount of support required, in the eastern part of the country it now appears probable that the introduction of rock bolting means that all tunnels will be supported to a much greater extent than formerly.

Investigations for Dams

The Board of Water Supply has constructed three large earth-fill dams and is about to begin construction of a fourth near Cannonsville, on the west branch of the Delaware River. About fifty years ago the New York State Water Supply Commission proposed construction of a dam at this site.[5] In 1945 the United States Engineer Corps studied it and made four test borings. In 1948 the Board of Water Supply began making test borings at this site.

At Cannonsville it is feasible to divert the stream through an open channel and conduit, rather than excavate a diversion tunnel through bedrock, and there is a suitable site for construction of an overflow weir founded on bedrock. The permeability and stability of the soil at the dam site have been tested in numerous ways. There are large deposits of soil suitable for use as impermeable core material and for permeable shell embankment.

The earth dams built by the board have been constructed by the rolled-fill method. Soils are placed in layers six inches in thickness and compacted by rolling with sheeps-foot rollers. The moisture content and density are controlled by laboratory tests.

Property Acquisition and Claims

In connection with the taking of property for water-supply use it often becomes necessary to make investigations and tests on alleged sand and gravel deposits or flagstone quarries. The geologist often is concerned with claims made against the city for various reasons. Geologic records are made to forestall and combat such claims.

The Soils Laboratory

A soils laboratory is an indispensable facility for water-supply work. It performs various functions, including (1) testing natural materials proposed for use on highway construction; (2) testing natural materials obtained from pits proposed as sources of concrete aggregate; (3) testing concrete aggregates as supplied for use on the work; (4) testing stone proposed for use as riprap or rubble masonry; (5) preparing concrete mixes; (6) preparing test cylinders of concrete and testing their compressive strength; (7) making Proctor compaction tests on soils; (8) making permeability tests on soils; (9) making shear tests on soils; (10) making consolidation tests on soils; (11) computing and tabulating records of hydrostatic pressure in completed dams; and (12) controlling earth embankment during dam construction.

Conclusion

My discussion has covered but one phase of water-supply work, that

of the engineering geology. There are many other phases: surveying, engineering design, engineering construction, the preparation of specifications and contracts, the acquisition of property, and legal problems. Together they represent a long-continued and tremendous amount of effort and work that must be done before an inhabitant of the city of New York can turn on his tap and find a ready supply of pure water.

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SECTION OF CHEMICAL SCIENCES

HIGHLIGHTS OF THE CHEMISTRY OF THE NEWER ANTIBIOTICS*

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The Sixth Annual Symposium on Antibiotics,[1] held in Washington, D.C., last fall, marked the thirtieth anniversary of Fleming's discovery of penicillin and the tenth anniversary of the introduction of broad spectrum antibiotics. Annual production of all antibiotics in the United States has now reached about 2½ million pounds. There is no doubt that with this vast consumption there has been marked improvement in the treatment and prevention of infectious diseases. Widespread use of these new drugs has been accompanied, however, by formidable medical problems. One of these is the urgent problem of resistant staphylococci and the current world-wide incidence of staphylococcal diseases. Among antibiotics used effectively on resistant microorganisms are several that are "killers" of the offending staphylococci: in order of efficacy, vancomycin, ristocetin, and kanamycin. Supplementing these are the "inhibitory" antibiotics: erythromycin, novobiocin, and oleandomycin; and, of course, this does not exhaust the list. None of these, however, is considered the answer to the serious present problem of "hospital staphylococcal diseases."

Allergic reactions to antibiotic therapy, which too often end in the death of the patient, constitute another problem that continues to concern the clinician.

The toxicity of antibiotics is seldom a major problem. However, control of certain infections requires the use of, for example, kanamycin or polymixin preparations. It has been pointed out that the toxic effects of a drug often parallel its therapeutic activity, and that this does not demand rejection of the drug, but merely greater precision in its use.

The role of the research chemist is humble when one considers the tremendous effort that is expended in discovering a new antibiotic, in establishing its clinical safety and usefulness, in maintaining adequate control over its use, and in assessing the hazards connected with its applications. The chemist uses his special skills in producing the antibiotic, in determining its chemical identity and, finally, perhaps, in modifying it in the hope that he can eliminate some of its undesirable biological effects and yet retain its therapeutic usefulness.

I shall deal now with some of the specific structure problems that have been solved recently and with the wide variety of structures found among the newer antibiotics.

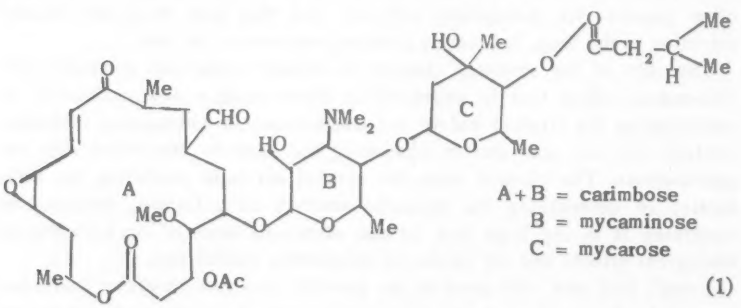
*This paper, illustrated with slides, was presented at a meeting of the Section on March 3, 1959.

During the last few years we have been hearing a great deal about a new class of natural product: the macrolide antibiotics. The common feature of these substances is a highly substituted, many-membered lactone system; all of them contain also a glycosidically bound amino sugar. Methymycin was the first of the class to yield to chemical studies; the structure was announced[2] by Djerassi late in 1956. There are a dozen or more closely related members of this macrolide family: among them pikromycin, magnamycin, erythromycin (therapeutically the most important), the spiramycins, and, currently under investigation,* oleandomycin.

There are two additional types of large-ring lactone among the recent antibiotics. The actinomycins and the recently reported etamycin are typical of the peptide lactones. Peptides usually have a free carboxylic acid group at one end of the polyamide chain, and a free amine group at the other. In these peptide lactone antibiotics, however, the carboxylic acid end group of the peptide chain appears as the lactone, and the amine group of the peptide is part of an amide linkage to a heterocyclic carboxylic acid.

The third type of many-membered lactone system is represented by the conjugated polyene antifungal antibiotic pimaricin, which has been studied elsewhere.† Pimaricin is the first of a considerable number of related substances to have its structure elucidated. Instead of the sprinkling of functional groups that we see in magnamycin and the other macrolides, pimaricin contains a cluster of oxygen-containing functional groups and a highly unsaturated chain of unsubstituted carbon atoms. The polyene macrolide structure has been demonstrated in one other member of the class, the antibiotic A-246.[3] Similar structures undoubtedly will be found among the many other polyene antifungal antibiotics.

Decision concerning the lactone system in magnamycin, FORMULA 1, was reached through study[4] of the behavior of reduced derivatives of

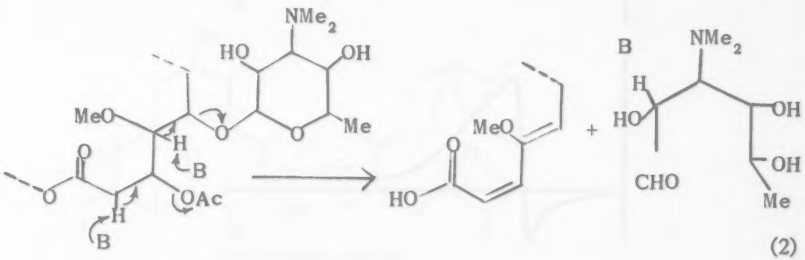


(1)

*By Charles Pfizer & Co., Inc., Brooklyn, N. Y.

†Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.

carimbose, obtained from magnamycin by mild hydrolytic cleavage of the neutral sugar, mycarose. Vigorous reduction of carimbose gave an octahydro derivative in which the only remaining unsaturation was the carbonyl group later identified as lactonic. Mild alkaline hydrolysis then gave dimethylamine and generated an α, β, γ, δ-unsaturated carboxylic acid system. The sequence of events shown in FORMULA 2 was suggested: base-catalyzed removal of a proton adjacent to the carbonyl group initiated



(2)

the reaction. After cleavage of the glycoside linkage, the dimethylamino group in the sugar moiety was in the base-susceptible position β– to the sugar carbonyl group, and was consequently lost as dimethylamine. Activation of the hydrogen on C-2 was required to account for this series of reactions; the difficultly reducible lactone carbonyl group was postulated. An elegant series of oxidative and hydrolytic reactions was then carried out to demonstrate the macrocyclic nature of the lactone system in magnamycin.

Structure studies[5] on the macrolide antibiotics methymycin and neomethymycin are of particular interest, since in them Djerassi has made application of his extensive studies in optical rotatory dispersion. Rotatory dispersion has been described as a "powerful physical tool with important contributions to problems of absolute configuration, location of carbonyls in polycyclic systems, analytical questions and detection of subtle conformational factors." The technique consists in plotting optical rotation against wave length in the range 245 to 700 mμ. The curves obtained (FIGURE 1) from a large variety of carbonyl compounds are of three general types: (1) plain, having a consistently negative or positive slope; (2) anomalous, single Cotton effect, in which appear one maximum value of the rotation and one minimum (both in a small range of wave lengths that also includes the absorption maximum of the substance); and (3) anomalous, multiple Cotton effect.

Important clues in Djerassi's studies were obtained from rotatory dispersion data. For instance, in neomethymycin (FORMULA 3) the acid degradation product anhydrocycloneomethynolide was found to have infrared maxima at 5.75 and 5.88 μ. Initially, these bands were assigned,

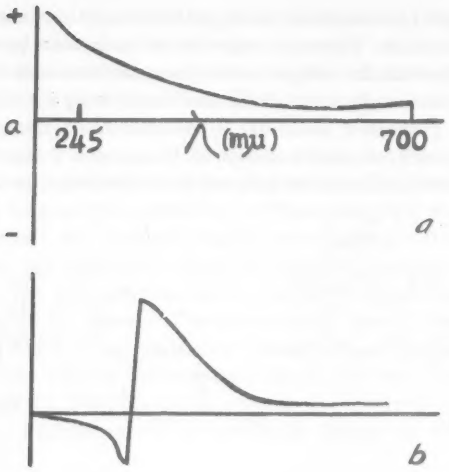
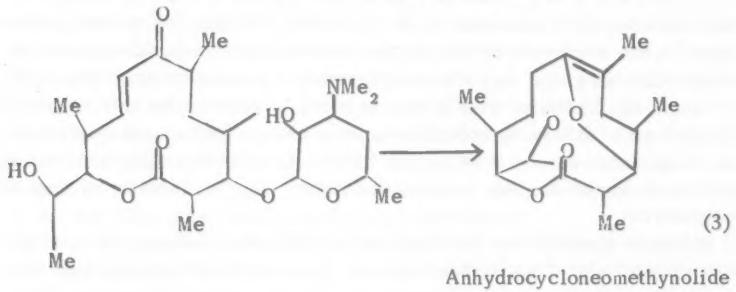


FIGURE 1. (a) Plain; (b) anomalous, single Cotton effect.



(3)

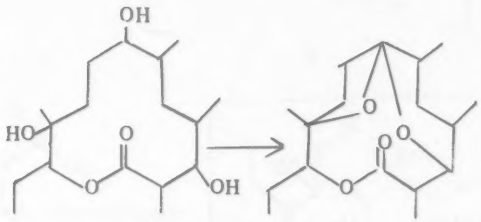
Anhydrocycloneomethynolide

respectively, to the lactonic carbonyl and the ketonic carbonyl, since these functional groups were known to be present in its precursors, with similar infrared absorption bands. However, the anhydro compound had plain positive rotatory dispersion, in contrast to the anomalous single Cotton effect curves observed in its precursors. Stability to lithium aluminum hydride of the 5.88 μ band in the anhydro compound confirmed the fact that it did not arise from a carbonyl group, and secure chemical evidence of its enol ether structure was subsequently obtained.

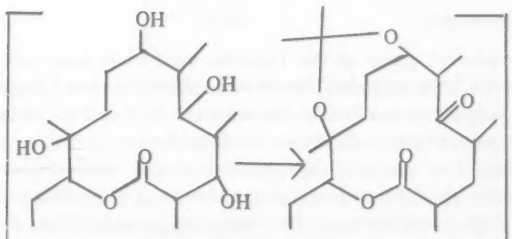
Pikromycin is another of the macrolide antibiotics, also an isomer of methymycin. It is known that the sugar residues in both methymycin and neomethymycin are on C-3. Pikromycin is believed to have a C-5-glycoside link to the amino sugar, but the possibility has existed that it is actually a C-3-glycoside, epimeric at C-3 with methymycin and neomethymycin. Djerassi has found, however, that while rotatory dispersion curves of

derivatives of methymycin and neomethymycin are very similar, those of corresponding derivatives of pikromycin are quite different. This suggests that the structural differences between these macrolides are more profound than simple C-3 epimerization.

Djerassi has investigated experimentally the isomerism of methymycin and pikromycin. Oxidation of tetrahydromethynolide (FORMULA 4) with chromic acid in acetone gave a bis-spiroketal; oxidation at C-7 preceded



(Methymycin series)



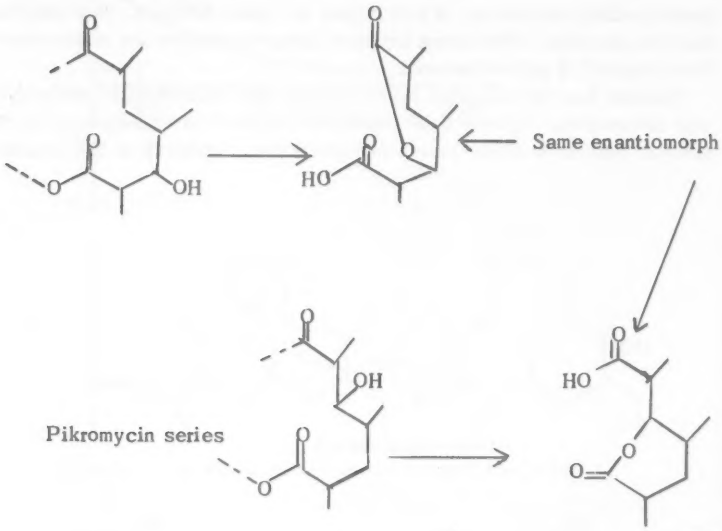
(Pikromycin series)
?

(4)

ketal ring closure. However, similar oxidation of tetrahydropikronolide led to an acetonide in which the originally glycoside-bonded hydroxyl group had been converted to a ketone group. Failure of this attempted interconversion indicated that pikromycin may actually be a C-5-glycoside, but the possibility remains that it is a C-3-glycoside that differs conformationally from methymycin.

During the neomethymycin studies, degradation to simple optically active fragments permitted assignments of absolute stereochemical configurations to C-4 and C-6. One of the intermediate degradation products was the lactonic acid (FORMULA 5), the carboxyl of which originated in C-1 of neomethymycin. The same enantiomorph of this acid has been obtained also from pikromycin. However, if the latter is a C-5-glycoside,

Neomethymycin series

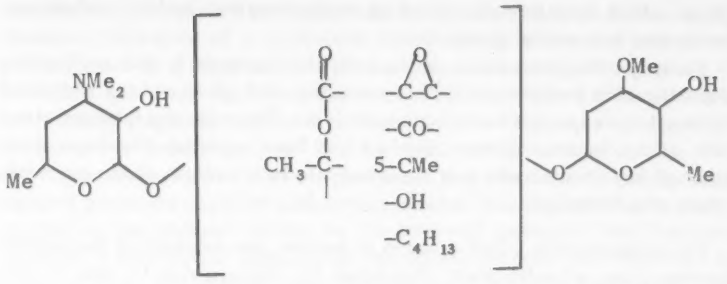


(5)

then the carboxyl group of the lactonic acid must have originated in C-7 of pikromycin. Consequently, the two antibiotics must be epimeric at C-4. Djerassi suggested the likelihood that the C-2 and C-6 asymmetric centers in these two antibiotics were also antipodes, and thus that the entire C-1 through C-7 portions of pikromycin and neomethymycin might be enantiomeric. He further speculated that these antibiotics may have their biogenetic origin in the union of 2 large fragments, rather than by successive fusions of 2- or 3-carbon units.

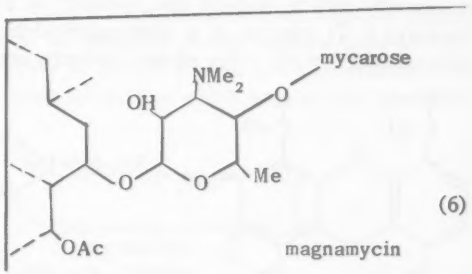
A preliminary report[6] on the study of the macrolide antibiotic oleandomycin has appeared recently. This antibiotic is currently in general use, often in combination with, for example, one of the tetracyclines, since there appears to be synergism, and also, possibly, a delay of development of resistant microorganisms. Oleandomycin (FORMULA 6) has the multiple functionality characteristic of all the macrolides.

Methanolysis under mild conditions yielded the neutral sugar oleandrose, which is known as the carbohydrate moiety of the plant glycoside oleandrin. The intact basic fragment from the methanolysis, desoleandomycin, was subjected to vigorous hydrolysis and found to yield the amino sugar desosamine, a degradation product that has also been obtained from several of the other macrolide antibiotics. Extensive decomposition took place during this hydrolysis; consequently the nucleus, free of sugar residues, could not be isolated. This was also the case with the macrolide magnamycin.



A B C

A+B = desoleandomycin
C = oleandrose
A = desosamine



(6)

magnamycin

In his studies[4] on magnamycin, Woodward had found that upon cleavage of the glycoside bond between mycarose and the amino sugar, the base strength of the nitrogen atom increased by about two *p*H units, apparently due to the electron-sink effect of the neighboring acetal oxygen atom in the intact antibiotic. This effect was not observed with oleandomycin; both the parent antibiotic and desoleandomycin were found similar in base strength. It was observed also that esterification of the free hydroxyl groups in oleandomycin resulted in a decrease of about two *p*H units in base strength, thus confirming the fact that the easily cleaved neutral sugar was linked directly to the oleandolide nucleus and not to the free hydroxyl in desosamine.

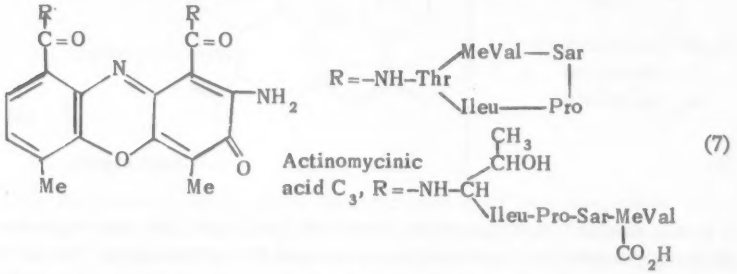
Alkaline hydrolysis of oleandomycin resulted in uptake of one equivalent of base, and no volatile acids could be detected. The amorphous reaction product was assigned zwitterionic functionality on the basis of the appearance of a 6.3-μ absorption maximum in the infrared. The substance could be esterified to yield a methyl ester that had normal ester carbonyl absorption near 5.8 μ. Evidence for the carboxyl group is thus secure.

Oleandomycin reacted slowly with periodic acid; no volatile carbonyl compounds were detected. However, the zwitterionic hydrolysis product consumed rapidly one mole of periodic acid and gave acetaldehyde. It was concluded that the saponification had also generated a new hydroxyl

group, which then formed part of an α-glycol system which, furthermore, terminated in a methyl group.

Thus, good evidence was at hand for the presence in the zwitterionic saponification product of both the carboxylic acid group and the additional hydroxyl, as expected for lactone hydrolysis. The size and intimate structure of the lactone system have not yet been reported. However, these data clearly demonstrate that oleandomycin is a member of the macrolide class of antibiotics.

The actinomycins, about a dozen in number, are members of the peptide lactone class of antibiotics. Structures for actinomycins C_3 and D were announced simultaneously in 1956 by Brockmann and his collaborators[7] in Germany and by Bullock and Johnson[8] in England. These substances (FORMULA 7) consist of a phenoxazine-3-one nucleus that holds two 16-membered lactone rings whose elements are amino acids. It is likely



(7)

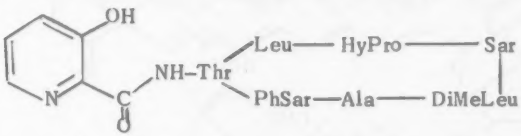
that all the actinomycins are similarly constituted and that they differ only in the amino acid composition of the peptide chains. A method has been developed for separating the intact peptide lactone systems before conventional amino acid sequence determination, and it has been shown that there are two identical lactone rings in each of the actinomycins thus far investigated.

It is not likely that these substances will ever be generally useful in view of their extreme toxicity, but they do have antitumor activity. The chemical studies that led to discovery of the nature of the chromophore in these bright-red crystalline substances are a classic in organic chemistry. In this review, we shall be limited to consideration of the evidence for the proposed lactone structures.

Actinomycin C_3 consumed 2 equivalents of alkali in about 4 hours; further consumption was much slower. The product, actinomycinic acid C_3, was a chromatographically homogeneous dibasic acid. The sodium salt no longer had the 5.7-μ infrared band of the parent antibiotic. The weakly basic group of actinomycin, titratable with perchloric acid, was still present, but no other basic groups could be detected. The 5 amino acids originally observed in actinomycin hydrolysates were still present in the acid. Actinomycinic acid, in contrast to actinomycin, was found to reduce

periodic acid, which indicated generation of hydroxyl groups in the saponification. Formation of a crystalline dimethoxy dimethyl ester completed characterization of this acid. At this point in the study, the presence of 2 lactone groupings in actinomycin was well established. The nature of the lactone ring was further determined by the observation that, while threonine was present in total acid hydrolysates of actinomycin C_3, this hydroxy amino acid was the only 1 of the 5 not detected in total hydrolysates of periodate-oxidized actinomycinic acid. This suggested, of course, closure of the lactone system on the hydroxyl groups of the threonine moieties. Conventional degradation procedures revealed the sequence of amino acids in the peptide chain, defined the C-terminal amino acid, and thus indicated the fine structure of the lactone systems.

The antibiotic etamycin[9] is the newest member of this class of peptide macrolactones. It appears to be effective chiefly against Gram-positive bacteria. Total acid hydrolysis of etamycin (FORMULA 8) yielded 3-hydroxypicolinic acid and 7 amino acids, 5 of which were readily identified.



(8)

The remaining 2 were the *N*-methylamino acids phenylsarcosine and *N*,β-dimethylleucine, which had not been observed previously in natural products. Degradative studies were carried out to establish their structures.

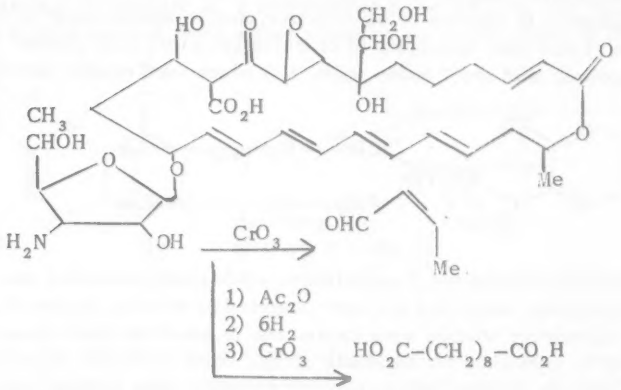
Etamycin contained no titratable groups other than the phenolic hydroxyl and the tertiary nitrogen of the picolinic acid residue. Alkaline hydrolysis gave etamycin acid; no second fragment containing an hydroxyl group could be detected, and no additional basic function had been generated. Etamycin acid, which contained all of the component amino acids of etamycin itself, was not relactonized in dilute acid or by treatment with dicyclohexylcarbodiimide. Under these conditions, small ring lactones would be expected to re-form easily.

Since only the threonine and hydroxyproline moieties contain hydroxyl groups, one of these must have been involved in the original lactone system. It was found that while both of these hydroxyamino acids were destroyed by chromic acid oxidation of etamycin acid, only the hydroxyproline suffered destruction when etamycin was similarly oxidized. In etamycin, then, the hydroxyl group in the threonine moiety must have been protected by virtue of the lactone linkage.

Since primary or secondary amine groups were not present in either etamycin or etamycin acid, it was apparent that the hydroxypicolinic acid was attached to the *N*-terminal amino acid of the peptide chain, and that the lactone group involved the terminal carboxyl group of the chain. In

order to apply a conventional peptide degradation procedure, it was necessary to reduce the picolinic acid residue, thus generating a secondary amine function, and to open the lactone ring with alkali. A series of seven successive applications of the Edman phenyl isothiocyanate procedure revealed the amino acid sequence in the octapeptide. Phenylsarcosine was defined as the *C*-terminal amino acid by the standard hydrazinolysis procedure. With these data, supported by results of studies on partial hydrolysates, the intimate structure of the peptide lactone etamycin was completely determined.

Pimaricin, an antifungal antibiotic studied in the Lederle laboratories,[10] has the unusual polyene macrolide structure shown in FORMULA 9. It was



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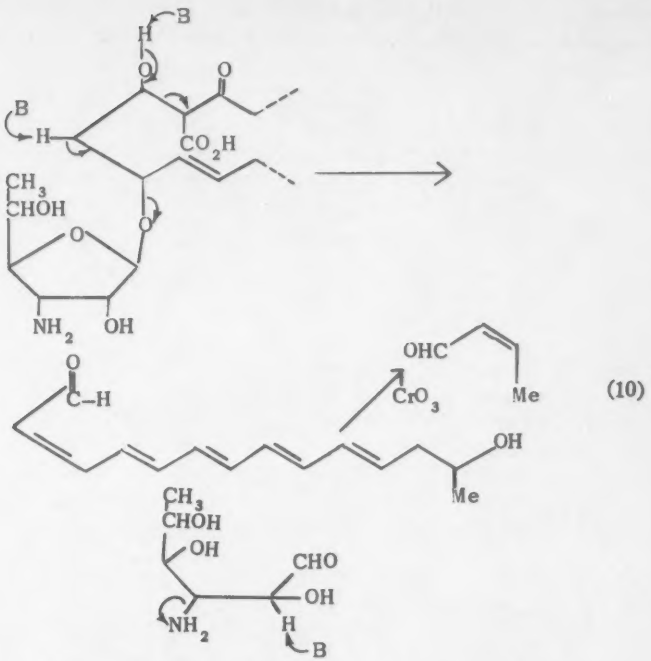
recognized as an α,β-unsaturated lactone from infrared and ultraviolet spectral data and from the observation that it consumed one mole of base to yield a single hydrolysis product.

When allowed to react with methanolic hydrogen chloride, pimaricin gave the amino sugar mycosamine, which has been obtained also from nystatin and amphotericin, two other members of this class of antibiotics. Crotonaldehyde was isolated after chromic acid oxidation of pimaricin; apparently the tetraene system was destroyed. Sebacic acid was obtained by chromic acid oxidation of a partially hydrogenated derivative of pimaricin. This indicated absence of alkyl substitution in the tetraene system.

Alkaline hydrolysis of pimaricin gave ammonia and a polyene aldehyde identified as 13-hydroxy-2,4,6,8,10-tetradecapentaeneal (FORMULA 10). The suggestion was made that this aldehyde resulted from dealdolization of a β-hydroxy ketone system as shown in FORMULA 10, and then successive β-eliminations, first of the sugar moiety β- to the newly formed



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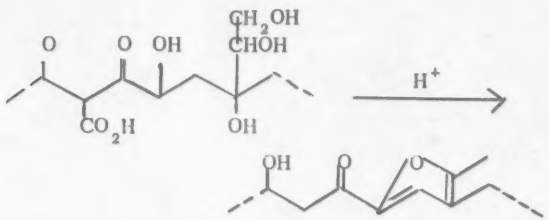
carbonyl, and then of ammonia from the β-amino aldose. The polyene aldehyde gave a much poorer yield of crotonaldehyde on oxidation than did pimaricin itself; hence the suggestion that the lactone system closure occurred on C-13 of the aldehyde moiety.

Dodecahydropimaricin, which still retained both the ketone and lactone groups, was found to contain one acetylatable hydroxyl in addition to those present in the parent compound. This was considered suggestive of epoxide functionality. Confirmation, and indication that the oxide linkage was α- to a carbonyl group, came from the observation that pimaricin liberated iodine from a solution of potassium iodide in acetic acid.

The carboxylic acid group was assigned to the position β- to the ketonic carbonyl, since pimaricin was readily decarboxylated in warm dilute sulfuric acid.

Periodic acid oxidation studies indicated that the three remaining oxygen atoms were present as hydroxyl groups. *N*-acetylpimaricin consumed one equivalent of oxidant rapidly and gave formaldehyde; a second equivalent of oxidant was consumed more slowly. A 1,2,3-triol system having one primary and one tertiary hydroxyl group was proposed. This unit was assigned to the ring carbon adjacent to the epoxide group, since *N*-acetyldodecahydropimaricin was found to dehydrate rapidly in hot dilute sulfuric

acid to yield a crystalline trianhydro derivative (FORMULA 11). This substance had the ultraviolet absorption characteristics of a furyl alkyl

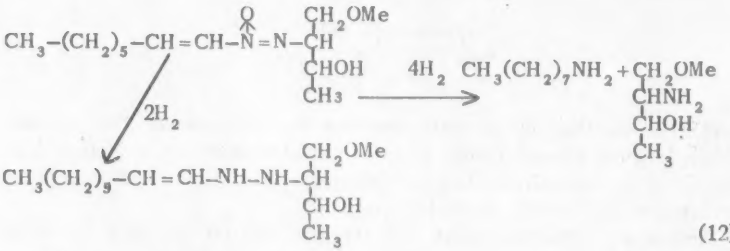


(11)

ketone. The reaction was interpreted as the dehydration of a carbohydrate-like system equivalent to a desoxyhexose.

Certain details of the structure of pimaricin remain to be confirmed, but the case for the polyene macrolactone system is conclusive.

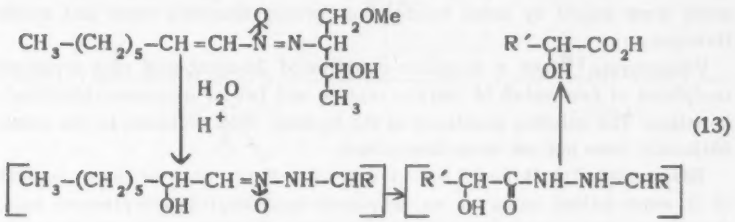
The antibiotic elaiomycin,[11] which has the unusual α,β-unsaturated azoxy structure shown in FORMULA 12, is also unique in that it is active



(12)

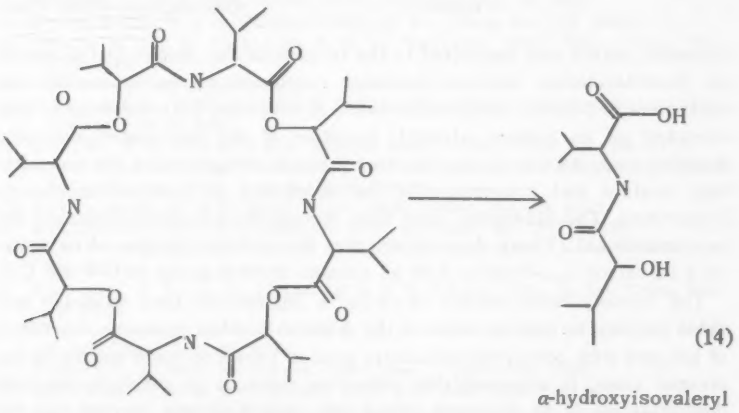
only against certain tubercle bacilli. The fundamental unit in its structure was discovered by hydrogenation studies. Analytical data permitted two double bonds in elaiomycin. Mild hydrogenation of the antibiotic resulted in two-mole uptake. A basic compound having one less oxygen atom was formed, and the suggestion followed that one equivalent of hydrogen had gone to hydrogenolysis of an *N*-oxide group. Vigorous hydrogenation resulted in four-mole uptake. Isolation of two primary amines indicated that one mole of hydrogen had been consumed in hydrogenolysis of a nitrogen-nitrogen bond. Together with spectral data, these observations required the azoxy group in elaiomycin, along with one other center of unsaturation. The two amines obtained upon vigorous hydrogenation of elaiomycin were identified as *n*-octylamine and 1-methoxy-2-amino-3-hydroxybutane; accordingly, the nature of the carbon skeleton was determined.

It was found that acid hydrolysis of elaiomycin gave α-hydroxyoctanoic acid (FORMULA 13). This behavior paralleled that of an α,β-unsaturated



aliphatic nitro compound, and the last detail of elaiomycin structure, the location of the carbon-carbon double bond, was assigned on this basis. Elaiomycin is seen to be the nitrogen analogue of an α,β-unsaturated nitro compound. It was suggested that the reaction involved addition of water to give the β-hydroxy compounds, followed by rearrangement to easily hydrolyzed hydroxamic acid derivatives. The mechanism of this reaction has not been established rigorously but, in general, highly oxidized nitrogen systems tend to undergo reduction at the expense of a neighboring methylene group.

Amidomycin[12] (FORMULA 14) is typical of a group of antibiotics that come close to defying any simple chemical classification: these are macrocycles in which amide and ester linkages alternate. Amidomycin is



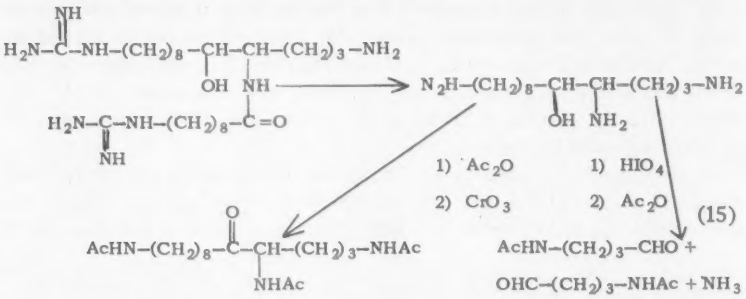
a stable, neutral compound that yielded D-valine and α-hydroxy isovaleric acid on vigorous acid hydrolysis. Mild saponification with methanolic

alkali resulted in uptake of four equivalents of base; the only substance detectable in the hydrolysate was recognized as α-hydroxyisovaleryl D-valine. The structure proposed was a macrocycle in which four of these units were joined by ester bonds, thus giving alternate ester and amide linkages.

Valinomycin[13] has a similarly-constituted 24-membered ring structure composed of two moles of lactyl-L-valine and two of α-hydroxyisovaleryl-D-valine. The relative positions of the hydroxy acid residues in the intact antibiotic have not yet been determined.

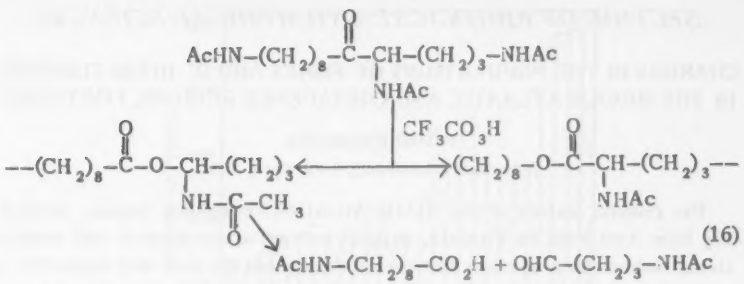
The enniatins A, B, and C are also in this class: each of them consists of 2 ester-linked units of an α-hydroxy-isovaleryl-*N*-methylamino acid, thus forming 12-membered rings having alternate ester and amide linkages.

A recently completed study[14] in our laboratories at Merck & Co., Inc. led to proposal of a structure for the antifungal antibiotic eulicin, FORMULA 15. Conventional hydrolyses led easily to the triaminoalcohol



eulamine, which was converted to the triacetylamino ketone in the course of characterization studies. Eulamine consumed extremely rapidly one equivalent of periodic acid, with release of ammonia. This behavior is that expected of an α-amino alcohol. Isolation of the anticipated aldehydic reaction products was finally realized through acetylation of the raw reaction mixture and conversion of the aldehydes to their dinitrophenylhydrazones. The aldehydes were then recognized as ω-aminononanal and ω-aminobutanal. These data established the carbon skeleton of eulamine as a 13-carbon α,ω-diamine with an α-amino alcohol group on C-4 and C-5.

The triacetylamino ketone (FORMULA 16) derived from eulamine provided the key to configuration of the α-amino alcohol grouping. Oxidation of ketones with peroxytrifluoroacetic acid is known to yield esters. In the present case, it appeared that either an ester or an aldehyde-ammonia derivative might be obtained. When this oxidation was carried out, the only substances isolated were the 9-carbon ω-acetylamino acid and the 4-carbon ω-acetylamino aldehyde, hydrolysis products of the aldehyde-ammonia derivative. This required that the amine group of the amino alcohol moiety be on the short carbon chain side of the hydroxyl group.



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The positions of the guanidine group in eulicin and the guanidinononanoyl moiety were readily established by periodic acid oxidation studies.

In conclusion, I call to your attention an article[15] carrying the provocative title "Doctor, Patient, Drug and Bug" by Ernest Jawetz of the University of California Medical Center, Berkeley, Calif. Very briefly, Jawetz's most significant recommendations were that there be, first, restrained, carefully considered use of all antibiotics and, second, special restriction on those antibiotics that are specifically effective against "hospital pathogens." Only through some universal rational program of this kind can we hope to achieve maximum effectiveness in our use of these invaluable drugs.

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CHANGES IN THE POPULATIONS OF FISHES AND IN THEIR FISHERIES IN THE MIDDLE ATLANTIC AND CHESAPEAKE REGIONS, 1930 TO 1955*

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The coastal waters of the Middle Atlantic-Chesapeake region, extending from New York to Virginia, support extensive commercial and recreational fisheries on species distributed throughout the area and subject to a common fishery of the bordering states. Probably the most drastic changes in the methods of fishing in this area occurred in the 1850s with the introduction of the pound net[1] and, in the late 1920s, with the development of the offshore otter trawl fishery, first only in winter[2] and then throughout the entire year.[3] Little information is available on statistics of the fisheries or the biology of the fishes in the period following the rise of the pound-net fishery, so that the effect of this gear on the fish and fisheries is not known. More information, although far from complete, is available on the fish and their fisheries from the inception of the offshore otter-trawl fishery to the present.[4-7] It is with this latter period, 1930 to 1955,** that we are concerned here.

The total annual catch since 1930 has been steadily increasing and, in 1955, it exceeded 1¼ billion pounds, or about 4 times the catch in 1930 (FIGURE 1). However, the great increase in the total catch was primarily a result of an increase in the catch of the menhaden, *Brevoortia tyrannus* and, to a much lesser extent, of the alewife, *Pomolobus pseudoharengus*. During the entire period the menhaden has been the principal species landed and has become increasingly important in the fisheries. Thus, from 1930 to 1939 the average annual catch of menhaden was more than 223 million pounds, or 57 per cent of the total average annual catch of all species; from 1940 to 1949 it was more than 465 million pounds, or 71 per cent of the total catch; and from 1950 to 1955 more than 809 million pounds, or 84 per cent of the total catch. Of the species remaining, the alewife comprises a substantial part of the catch, especially in recent years. In the years 1930 to 1939, the average annual landing of alewives was about 23 million pounds, or 14 per cent of the average annual landing of all fishes other than menhaden; from 1940 to 1949 it was more than 20 million pounds, or 11 per cent; and from 1950 to 1955 more than 32 million pounds, or 21 per cent.

Both the menhaden and alewife have been little affected, at least directly, by the development of the otter-trawl fishery. The menhaden is an in-

*This paper, illustrated with slides, was presented at a meeting of the Section on March 9, 1959.

**There is a lag in publication of fisheries statistics. This report was completed when 1955 data were the most recent available. Data for 1956 have since been released and examined and, since they did not change the findings presented here, are not included.

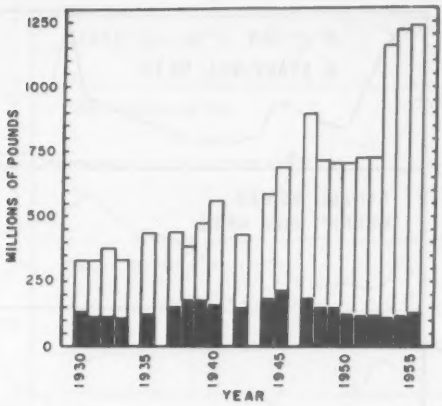

FIGURE 1. Annual landings of fishes from New York to Virginia, 1930 to 1955. Base line to top of white rectangle represents total landings of all species. Base line to top of black rectangles represents total landings of all species exclusive of menhaden and alewives.

dustrial species that is processed into fish meal and oil. From 93 to 99 per cent of the menhaden landed each year since 1930 were taken by special menhaden purse seines; the large increase in catch is attributable not only to an increase in abundance, but also to technological improvements in fishing and handling. Larger engines enable the boats to get to and from the fishing grounds faster. Surface schools are located by airplanes and reported by radio to the vessels. Echo sounders and asdic find fish beneath the surface. Suction pumps are used to unload fish from the holds of the vessels and more recently, to remove the fish from the seine itself. A power block has been substituted for manpower in lifting the net. The alewife caught in the greatest numbers in Chesapeake Bay is a low-priced food fish that has been landed in larger quantities in recent years, most probably as a result of increased marketability since World War II. The bulk of the catch is taken by pound nets.

After this brief reference to the menhaden and alewife which, because of their methods of capture by purse seine and pound nets, respectively, could not be affected directly by the spectacular development of the offshore otter-trawl fishery, let us turn our attention to the remaining fishes and their fisheries. During the period 1930 to 1955 there was a drastic change in the manner in which fish were caught in the Middle Atlantic-Chesapeake region (FIGURE 2). In the early 1930s over 55 per cent of the catch was taken by pound nets and less than 20 per cent by otter trawl. By the mid-1950s the positions were reversed: less than 15 per cent was taken by pound nets and more than 60 per cent by otter trawls. In addition, two other gears, the haul seine and, to a lesser extent, the drift-gill net,

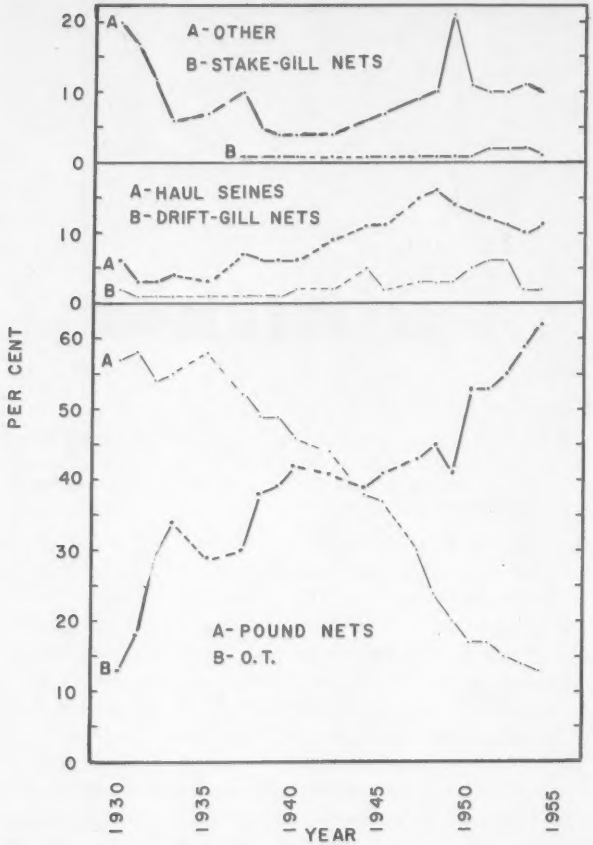

FIGURE 2. Percentage of fish exclusive of menhaden and alewives landed by various types of gear, New York to Virginia, 1930 to 1955.

caught a greater percentage of fish than formerly. The changes in the method of fishing are reflected in the changes in the number of various gears used (FIGURE 3). The number of pound nets fell almost 60 per cent. The number of small otter trawlers, presumably boats less than 5 net tons, remained constant, but the number of large otter-trawl vessels, the components of the offshore otter-trawl fleet, increased almost 400 per cent. The yardage of haul seines used increased from 250 to 400 per cent, and the yardage of gill nets about 400 per cent. Obviously, in recent years there has been a marked rise in the number of active gears that seek out the fish, that is, otter trawls, haul seines, and gill nets, and a fall in the number of static gears that wait for the fish to appear, such as the pound nets.

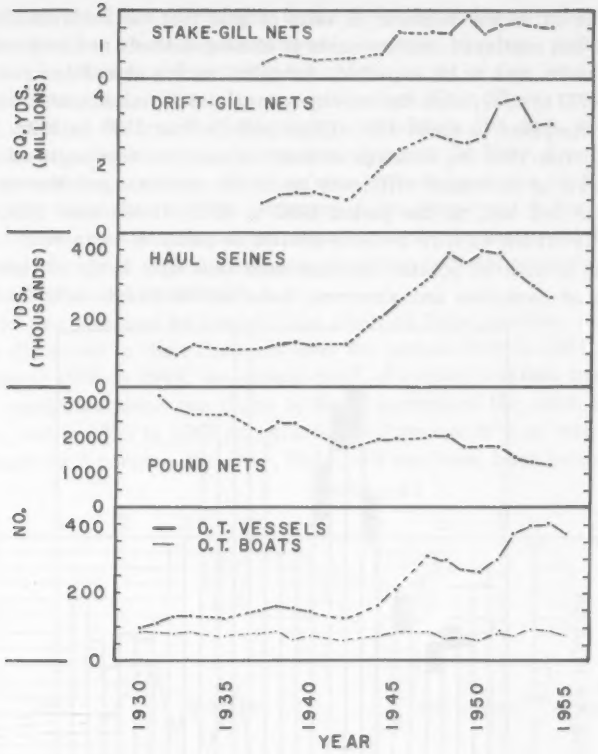

FIGURE 3. Quantities of the various major types of gear used in the fisheries, New York to Virginia, 1930 to 1955.

Not only has the number of the more active gears grown, but their efficiency has also increased. The offshore otter-trawl vessels are larger than formerly, enabling them to fish farther offshore and in more inclement weather. They are powered with larger engines that drive them faster to and from the fishing grounds and permit them to tow over a greater area at a higher speed. Echo sounders assist in finding the fish and fishery grounds, as do the radio compass, loran, and other recently developed aids to navigation. New types of nets have been developed, as for example, the balloon trawls that are specially adapted to catch certain species of fishes. The smaller otter-trawl boats have also increased in efficiency by utilizing some of the technological improvements adopted by the larger otter-trawl vessels. In both the haul seines and drift-gill nets a major increase in efficiency has been attained by increasing the average size of the net, in the former almost twice and, in the latter, more than three times.

As a result of the increase in units of gear that can actively seek out the fish and continued improvements in fishing methods and technology, a rise in catch was to be expected. Actually, such a rise did occur in the period 1937 to 1949, when the average annual catch was almost 176 million pounds compared to about 126 million pounds from 1930 to 1935. Nevertheless, from 1950 on, although at least as many or more units of gear of comparable or increased efficiency as in the previous period were used, the catch fell and, in the period 1950 to 1955, it averaged 122 million pounds (FIGURE 4). How can this decline in catch be explained?

First, it must be pointed out that more than fifty kinds of fishes, exclusive of menhaden and alewives, make up the Middle Atlantic-Chesa-

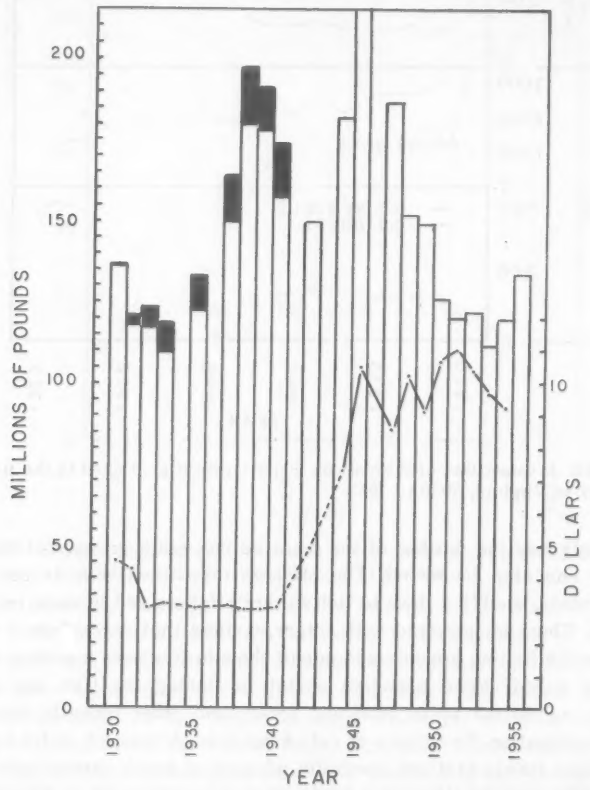

FIGURE 4. Annual catch of fish exclusive of alewives and menhaden, New York to Virginia, 1930 to 1955. Black rectangles are the catch taken by Massachusetts otter trawlers in Virginia waters and erroneously credited to Massachusetts. Broken line indicates the average price per pound of all fish exclusive of alewives, menhaden, and the Massachusetts catch.

peake catch. Analysis of changes in the fisheries and populations of all of these species would be an extremely complex problem, perhaps impossible of solution, and certainly beyond the scope of this limited presentation. Fortunately, 5 species comprise about 50 to 55 per cent of the catch, namely, the croaker, *Micropogon undulatus;* the gray sea trout, *Cynoscion regalis;* the scup, *Stenotomus chrysops;* the fluke, *Paralichthys dentatus;* and the sea bass, *Centropristes striatus* (FIGURE 5). Furthermore, these same fish are the mainstay of the otter-trawl fishery, while three of them have been important constituents in the pound-net fishery. Thus, any change in their fisheries or populations should certainly have a marked effect on the total catch.

Let us see what can be learned from study of these species. A radical change did occur in their fisheries over the period 1930 to 1954. During the interval 1930 to 1949, the annual catch of croaker and sea trout combined constituted about two thirds to three quarters of the catch of the 5 species, but in 1950 to 1954 comprised less than one fifth of their catch. The remaining 3 species, the scup, fluke, and sea bass, have become more

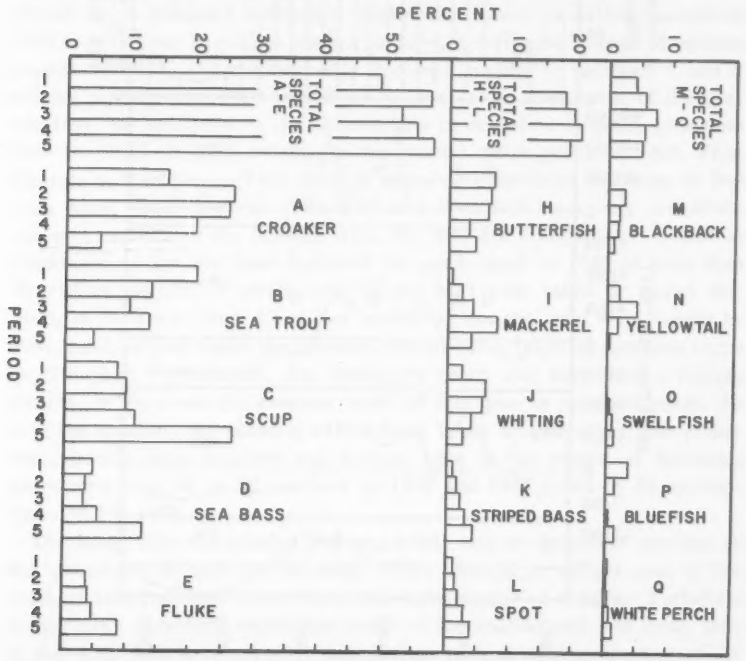

FIGURE 5. Average percentage composition by 5-year periods of some important species in the catch of all fishes exclusive of menhaden and alewives, New York to Virginia. Period 1, 1930 to 1934; period 2, 1935 to 1939; period 3, 1940 to 1944; period 4, 1945 to 1949; period 5, 1950 to 1954.

important in the catch in recent years. An explanation for this shift in the relative importance of the 5 species in the catch and its subsequent effect on total catch can be found in data on the individual species.

The average annual catch of croakers in 1930 to 1933 was about 20 million pounds. It doubled in the period 1935 to 1949, and then fell to about 7 million pounds in 1950 to 1955 (FIGURE 6). This species was the mainstay of the pound net fishery in the Chesapeake region and an important part of the New Jersey pound net fishery. In the early 1930s about 80 per cent of the catch was taken by pound nets. The center of population of the croaker appears to be the Chesapeake region. In years of high population level it expands north to New York. Some idea of the changes in abundance since 1930 is indicated by the percentages of deviation in catch each year

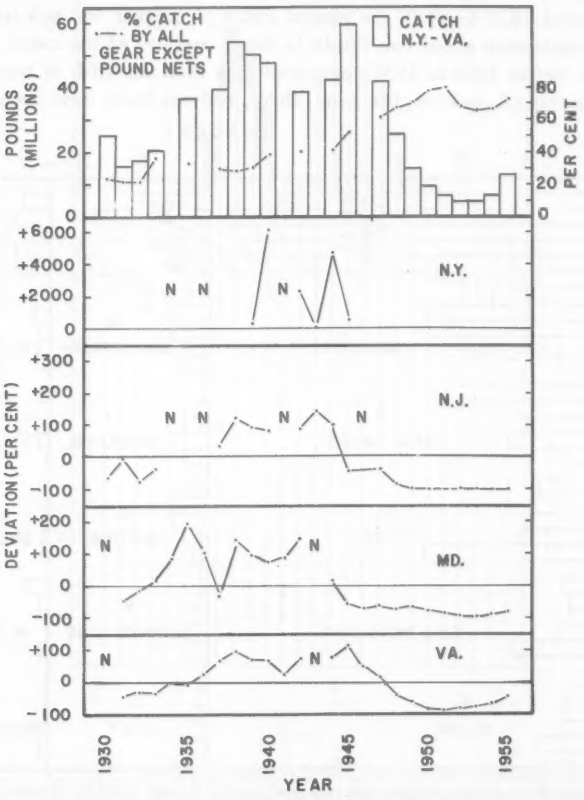

FIGURE 6. Total catch of croakers, New York to Virginia, and the annual percentage deviation of the catch per pound net from the average catch per pound in various states for the period 1931 to 1955. *N* means no data available.

from the 1931 to 1955 average of the annual catch per pound net. From Virginia to New York the level of abundance was low in the early 1930s. It rose rapidly in the late 1930s and remained high until the mid 1940s. Then abundance declined abruptly to a lower level than in the early 1930s. The abrupt decline in abundance followed a peak catch in 1945 of almost 60 million pounds of croakers, of which only about 50 per cent was taken by pound net. More than 15 million pounds were taken by otter trawl. The otter-trawl catch was 1½ times as great as the highest catch made by this gear in previous years. In addition, almost 14 million pounds of fish were caught by haul seine, or 7 to 14 times the quantity caught by this gear in the early 1930s. As the abundance decreased, the quantity of croakers taken by gear other than the pound net, mostly otter trawl and haul seine, rose from about 50 per cent in 1945 to 60 to 80 per cent from 1947 to 1955.

Like the croaker, the gray sea trout was also an important species in the pound net fishery. In the early 1930s, from 80 to 90 per cent of this fish was captured by pound nets (FIGURE 7). The main center of population of the gray sea trout appears to be the Chesapeake region. The total annual catch followed a trend of decline from over 32 million pounds in 1930 to less than 20 million pounds in 1944. It then rose to over 36 million pounds in 1945, and subsequently declined rapidly to between 4 and 8 million pounds from 1950 to 1955. Changes in the abundance of the gray sea trout are indicated by the percentages of deviation in catch each year from the 1931 to 1955 average of the annual catch per pound net. From Virginia to New Jersey,* the level of abundance remained at a more or less even level from 1940 to 1945-1947 and then fell to a very low level, where it remains at the present time. As with the croaker, the decline in abundance of the sea trout followed the peak catch in 1945 of more than 36 million pounds, of which only 52 per cent were taken by pound net. Also, in 1945 more than 12 million pounds of the sea trout were caught by otter trawl, or four times the greatest annual catch taken in previous years by this gear. Furthermore, the haul-seine catch was more than 2 million pounds, or 1½ times the greatest catch of this gear in previous years. As with the croaker, the quantity of sea trout taken by gear other than pound nets, mostly otter trawlers and seines, rose in the period of declining abundance from 40 to 50 per cent in 1945 and 1947 to 60 to 70 per cent from 1952 to 1955.

The scup, like the croaker and sea trout, was an important species in the pound-net fishery. In the early 1930s from 35 to 45 per cent of the catch of this fish was caught by pound nets, mostly in the New York-New Jersey area. However, unlike the catch of the croaker and sea trout, that of the scup has been steadily increasing from an annual average of 10

*New York is not included because it comprises a mixture of both the Chesapeake-centered population of fish and a locally spawned population; the complications arising from this fact cannot be discussed here.

million pounds in 1930 to 1933 to 16 million pounds in 1935 to 1947 and to 30 million pounds in 1948 to 1955 (FIGURE 8). However, the larger catch does not reflect an increase in abundance. If anything, data on the annual catch per pound net would indicate a somewhat lower abundance in recent years. The larger catch is a result of a more intensified fishery for scup, partly because of increased marketability. The scup is a less expensive fish than the croaker or sea trout and, in earlier years, only the larger fish could be sold. More recently, small fish have become acceptable to the consumer, particularly in the southern states. Another reason for the more intensified fishery for scup is the present low level of abundance of the croaker and sea trout. The otter-trawl fleet has been forced to turn to the scup as a replacement species, with the result that in 1955 more than 94 per cent of the scup catch was taken by otter trawl.

Information on the catch of fluke is available only since 1937. Previous

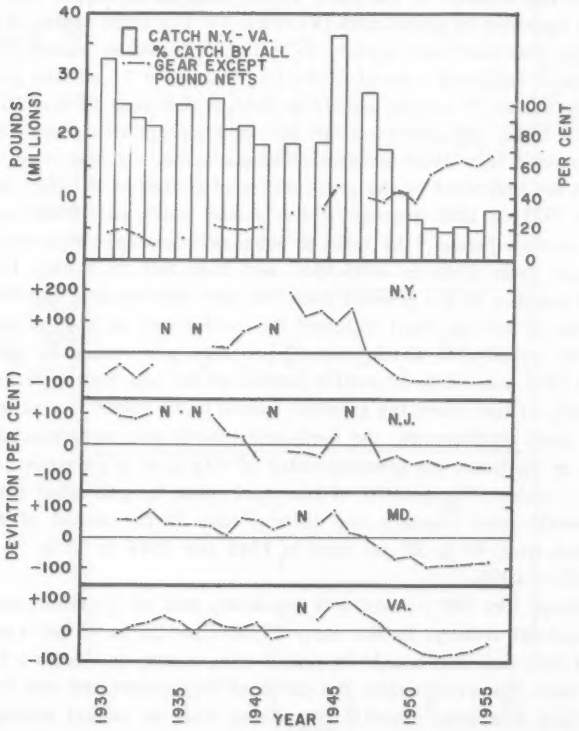

FIGURE 7. Total catch of gray sea trout, New York to Virginia, and the annual percentage deviation of the catch per pound net from the average catch per pound net in various states for the period 1931 to 1955. *N* means no data available.

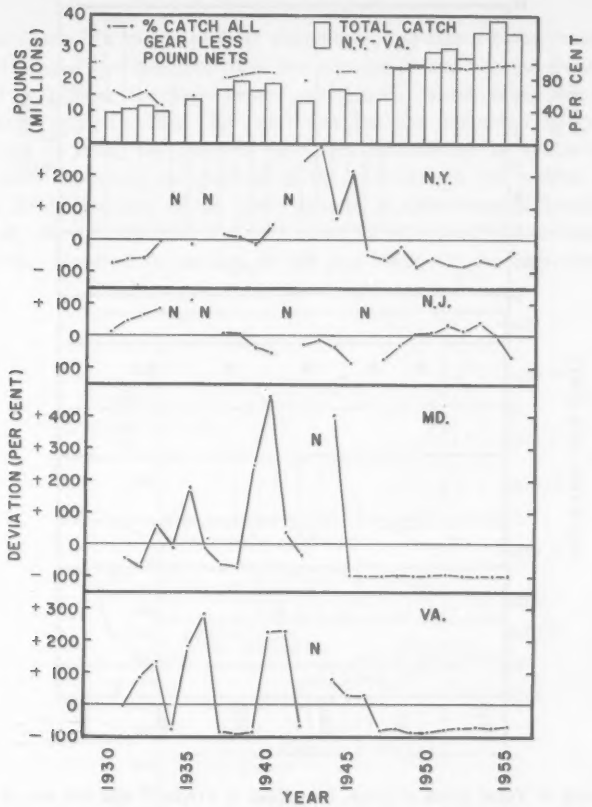

FIGURE 8. Total catch of scup, New York to Virginia, and the annual percentage deviation of the catch per pound net from the average catch per pound net in various states for the period 1931 to 1955. *N* means no data available.

to that year the catches of all species of flounders, including the fluke, were combined. The bulk of the fluke caught, 90 to 95 per cent, is taken by otter trawl; the remaining 5 to 10 per cent by pound net. During the period 1937 to 1951, the annual catch ranged mostly from 7 to less than 10 million pounds, but rose in 1952 to 1955 to about 10½ to 13½ million pounds (FIGURE 9). Again, as with the scup, the recent rise in the catch of fluke is not due to an increase in abundance, but to greater fishing activity for the species. Information on abundance, as indicated by the annual catch per pound net, at first glance appears to support this statement only partly. While the abundance level as measured by the New York and New Jersey fisheries has fallen and, in the Maryland fishery, has remained stable, it has increased in the Virginia fishery. A probable explana-

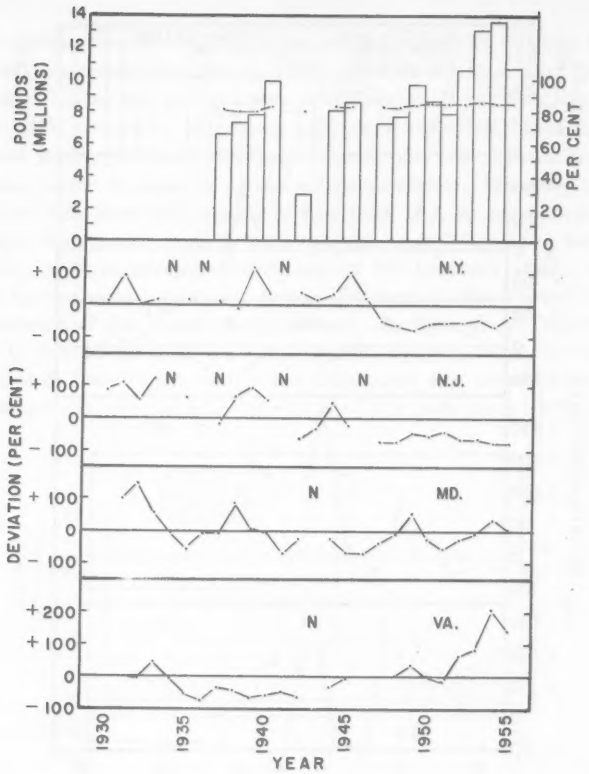

FIGURE 9. Total catch of fluke, New York to Virginia, and the annual percentage deviation of the catch per pound net from the average catch per pound net in various states for the period 1931 to 1955. *N* means no data available.

tion for the differences between the northern and southern fisheries is that, in the north, young fish are uncommon and, by law, small fish less than 14 inches in length are not marketed. Consequently, the decrease in abundance in the north actually reflects a reduction in the size of the population of fluke more than 14 inches long. In the south, where young fish are common and are allowed to be sold, price determines the quantity of small fluke discarded or marketed, and thus the catch per trap is influenced by economics rather than actual abundance of fluke.

The last species to be considered, the sea bass, is caught by 2 major gears, the otter trawl and the fish pot. Commonly, 70 to 80 per cent of the catch is taken by otter trawl, and 20 to 30 per cent by pot. Although the catch has increased from a range of 4 to 7 million pounds in 1930 to 1947 to 10 to 21 million pounds in 1948 to 1955, this rise in catch is not

to be attributed to an increase in abundance, but rather to increased fishing activity for the species. That the abundance has not increased is indicated in data on the New Jersey pot fishery, which takes most of the pot catch (FIGURE 10). The catch per pot has remained fairly stable from 1935 to 1955, the interval for which information is available. The total catch has gone up because of the increase in the number of pots fished. Also, because of the lower levels of abundance of croakers and weakfish, some of the time formerly spent by otter trawlers seeking those fishes was diverted to pursuit of the sea bass. Of the five species of

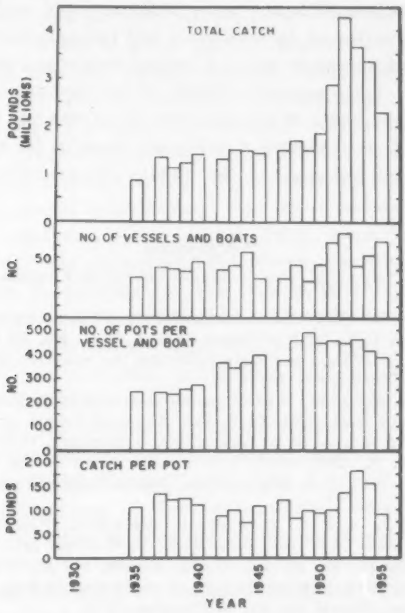

FIGURE 10. Statistics of the sea bass pot fishery in New Jersey, 1930 to 1955.

fishes discussed, the sea bass appears to be the only one in which the level of abundance has not declined. It is also the only species that inhabits a rough bottom not as completely accessible to the otter trawl as the smoother bottom on which the other species are commonly found.

From the brief description of what has happened to five of the more important food fishes in the Middle Atlantic-Chesapeake region, some conclusion can be drawn as to the reason for the decrease in total catch in the 1950 to 1955 period. Evidently, a decline in abundance in four of the five species, especially in the croaker and sea trout, is partly responsible

for the lower total catch. Incidentally, although space precludes a discussion of the other species of fish, I may mention the fact that the catches of butterfish, whiting, blackback flounder, yellowtail flounder, and mackerel also fell and that there is some indication that they also are less abundant than formerly.

While this fragmentary picture of the changes in the populations of fishes and their fisheries in the Middle Atlantic-Chesapeake region is crude, as are the data on which it is based, nevertheless it does indicate the possibility that detrimental changes are occurring in the fisheries. Certainly, in view of these possibilities, the fisheries in the area should be carefully watched. Although some biological and statistical investigations are being carried on by both state and federal conservation agencies, these studies unfortunately are of a limited nature and poorly coordinated. It is regrettable that a general attitude of indifference or perhaps lack of appreciation of the value of our fisheries resources has thus far prevented establishment of an expanded, coordinated program for a continuous study of the fishes and fisheries in the Middle Atlantic-Chesapeake regions.

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DIVISION OF INSTRUMENTATION

PROBLEMATIC ASPECTS IN ANALYSIS OF COMPLEX MEDIA BY MEANS OF THE AUTOANALYZER*

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Squibb Institute for Medical Research, New Brunswick, N. J.

A recent development in instrumentation has made available a means for continuous automatic colorimetric analysis on a large-scale basis.[1,2] The instrumentation system seen in FIGURE 1 is capable of carrying out automatically a number of steps required in colorimetric analyses, for example, sampling, diluting, adding reagents, sample purification, color mensuration, and recording. The controlling component of the system is a proportioning pump. The pump is designed continuously to impel 8 separate streams (fluids or gases) simultaneously by the action of rollers on polyvinyl chloride tubing. It consists of two parallel steel roller chains with spaced roller thwarts that travel at constant speed and bear continuously against a spring-loaded platen. When the plastic tubes are inserted between the rollers and the platen, the fluids in the tubes advance at a rate proportional to the square of the diameter of the tubes. Hence, various ratios of sample, diluent(s) and reagent(s) are achieved. Other components of the system are a sampling unit, a continuous concurrent dialysis unit, a heating bath, mixing coils, a dual beam type colorimeter with a continuous flow cell, and a recorder. The various components are interconnected with polyvinyl chloride tubing according to the requirements of the analytical method. Samples can be analyzed at the rate of 20, 40, or 60 per minute.

The earliest applications of the Autoanalyzer† were in the area of clinical chemical analysis. Various normal and pathological constituents of blood and urine were satisfactorily determined after partial purification of the desired constituent in the continuous dialysis unit. No interfering blanks were encountered in such diverse analyses as those for glucose, urea nitrogen, alkaline phosphatase, chloride, and phosphates even though the reagents used were relatively nonspecific.

In the large-scale production of antibiotics, rigid process control measures are required at all stages of fermentation and purification to ensure both quality of product and economy of operation. Hence, the analysis of large numbers of samples is encountered on a daily routine basis. Attempts to automate the steps in the chemical analysis of antibiotics have not been completely successful. This has been due

*This paper, illustrated with slides, was presented at a meeting of the Division on March 17, 1959.

†Trademark, Technicon Corp., Chauncey, N. Y.

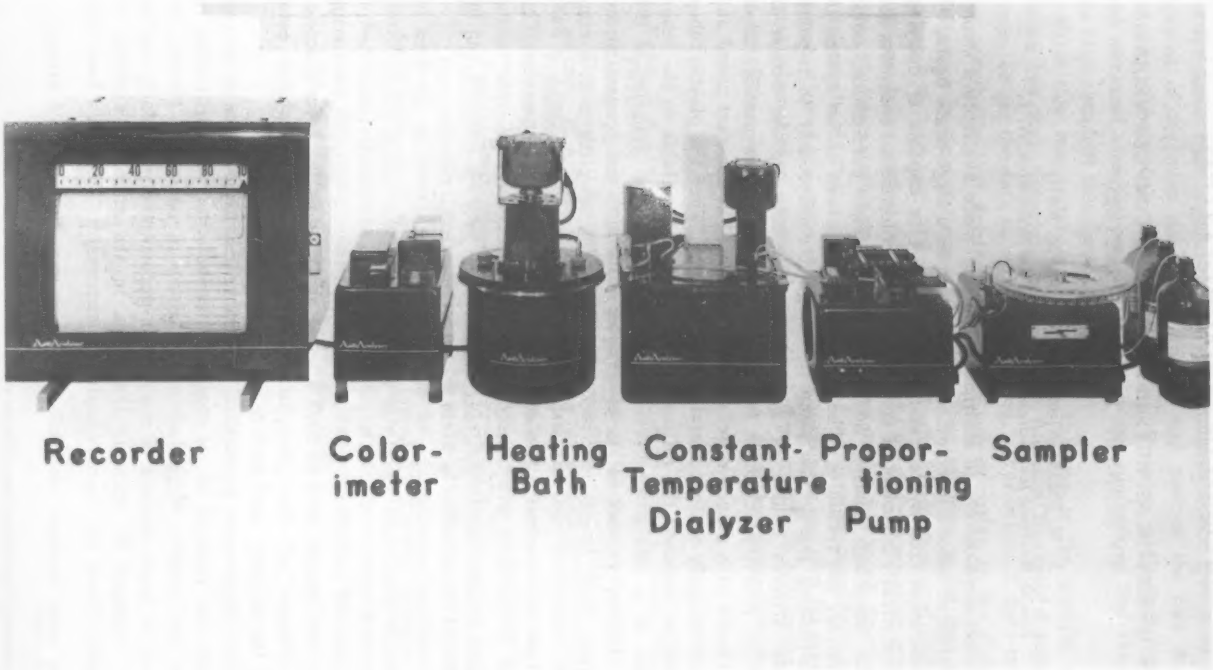

FIGURE 1. Technicon Autoanalyzer.

principally to the difficulties involved in the analysis of complex fermentation media and to the relatively low concentration of the constituent to be determined. No instrumentation systems were available that could automatically perform the many sequential steps necessary in the analysis. For example, a method for the determination of penicillin in general use in industry involves a number of manipulations.[3,4] Isopropyl alcohol is added to an aliquot of fermentation media to precipitate proteinaceous material. The supernate is separated by centrifugation and two aliquots are measured, one for a blank determination, the other for the unknown. A measured amount of standard, buffered iodine solution is added to each. Next, a measured amount of aqueous penicillinase is added to the unknown, and an equal amount of distilled water to the blank. Both are permitted to stand at room temperature for twenty-five minutes, during which time the penicillin is converted to penicilloic acid by the action of the enzyme. Excess iodine in each is then determined, using standard thiosulfate solution and a starch indicator. The difference in iodine uptake between the unknown and the blank represents penicillin concentration. The elapsed time between the actual sampling and relay of information approximates 50 minutes.

Since iodine can be measured colorimetrically as well as titrimetrically, the Autoanalyzer system was adapted to the iodometric penicillin assay. The instrument flow diagram is seen in FIGURE 2. The numbers adjacent

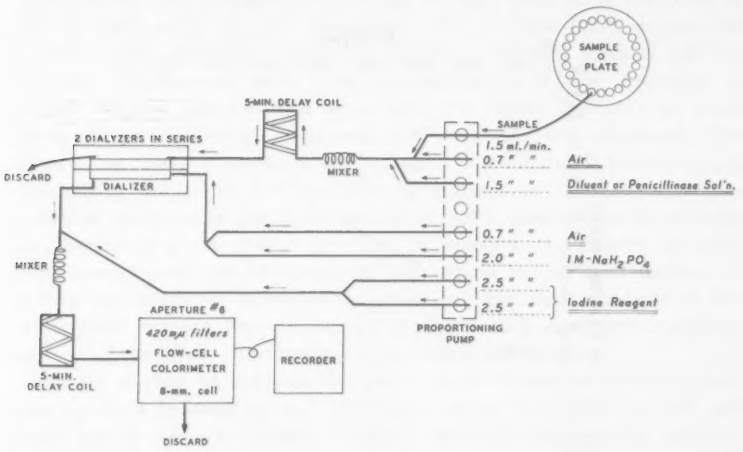

FIGURE 2. Flow diagram of iodometric penicillin method.

to the tubes refer to the volume pumped per minute. The centrifuged sample of broth is picked up through the sampling unit, segmented by air, and mixed with an aqueous stream of penicillinase. (Mixing is

accomplished in the Autoanalyzer by pumping convergent streams through appropriate lengths of horizontal glass helices.) This mixture is then passed through a five-minute time-delay coil wherein a portion of the penicillin is converted to penicilloic acid. This penicilloic acid stream is passed into the continuous dialyzer and dialyzed against a concurrent stream of aqueous *p*H 6.5 phosphate buffer that picks up a portion of the acid. The diffusate emerging from the dialyzer is mixed with a stream of buffered iodine solution, passed through a five-minute time-delay coil to allow for iodination and then into the colorimeter where the percentage of transmission at 420 mμ is recorded. The blank is run in the same manner except that distilled water is used instead of penicillinase solution. Potencies are calculated by converting recorded optical densities of blank and unknown into potencies from a standard curve and subtracting the blank from the unknown. A typical standard curve for aqueous penicillin G standards is seen in FIGURE 3. Recorded transmittancies of unknown fermentation samples and the corresponding blanks are shown in FIGURE 4.

The earliest trials with this method gave reasonably satisfactory results in most cases. However, as broader concentration ranges and widely varying fermentation media were encountered, it became evident that the method had a number of limitations. Valid analyses of many fermentation media encountered were confined to rather narrow ranges of

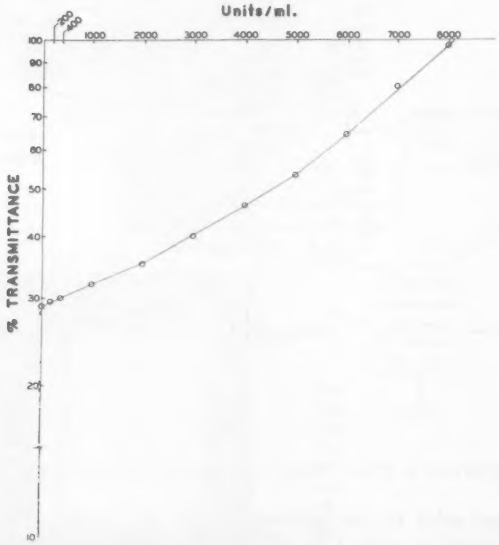

FIGURE 3. Beers law plot of optical densities - penicillin standards.

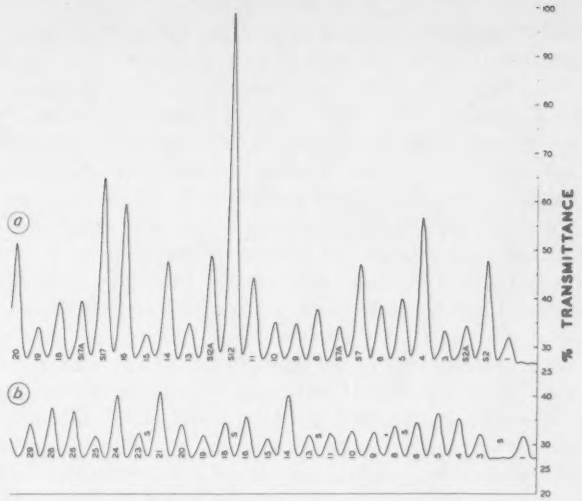

FIGURE 4. Recording of penicillin broth, processed samples, and the corresponding blanks. (*a*) Samples and standards; (*b*) blanks.

concentration and required the construction of a standard curve that approximated these ranges. The actual working limit is of the order of 400 to 1600 units per ml. of sample. Outside this limit, pronounced curvature was observed; hence the samples required dilution and reanalysis. Furthermore, with some fermentation media the responses of serially diluted samples with water were not linear, and lack of parallelism between blanks, unknowns, and standards was observed. The reasons for these phenomena are not clearly understood. However, one contributing factor is that the rate of dialysis of the enzymatic degradation products of penicillin is not directly proportional to concentration and is a curvilinear function. In addition, the effects of other broth constituents on all steps in the method varied from medium to medium and could not easily be assessed or controlled. Attempts to use the method without the continuous dialysis step were unsatisfactory because of the gross interferences from broth constituents.

Another method of analysis for penicillin is based on a colorimetric reaction with hydroxylamine.[5] Penicillin reacts with this reagent and ferric ion to give a colored complex while its degradation product, penicilloic acid, does not. Therefore, an estimate of penicillin concentration may be obtained by the difference in color response before and after enzymatic decomposition. The chemistry of this method was adapted for automatic operation on the Autoanalyzer and included the continuous dialysis step. As previously stated, the dialysis step resulted in a curvilinear response and a limited valid analysis range. Hence, it

was decided to study this method without the use of the dialyzer. This requires that the fermentation samples be centrifuged before introducing them into the sampling unit. The flow diagram for this analysis is shown in FIGURE 5. The numbers adjacent to the tubes again refer to volume pumped per minute. For each estimate of penicillin potency a blank and an unknown determination are needed. In operation, the unknown is picked up from the sampling plate, segmented by air, and diluted with a stream of distilled water. This mixture is pumped through a standard mixing coil and then through a five-minute time-delay coil. After this period it is joined by a stream of unsegmented hydroxylamine reagent and passed through a second mixing coil. After mixing, it is met by a stream of ferric ammonium sulfate reagent, mixed again, and passed through a three-minute time-delay coil to develop color. The colored stream enters the flow cell of the colorimeter where the transmittancy is measured at 480 mμ and recorded. The flow system for the blank determination is exactly the same, except that an aqueous penicillinase solution is substituted for distilled water in the initial dilution. In this method, it is necessary to separate all samples (unknown and blanks) with sample cups of distilled water to decontaminate the system adequately. Ten determinations of penicillin potency can be made with this system each hour.

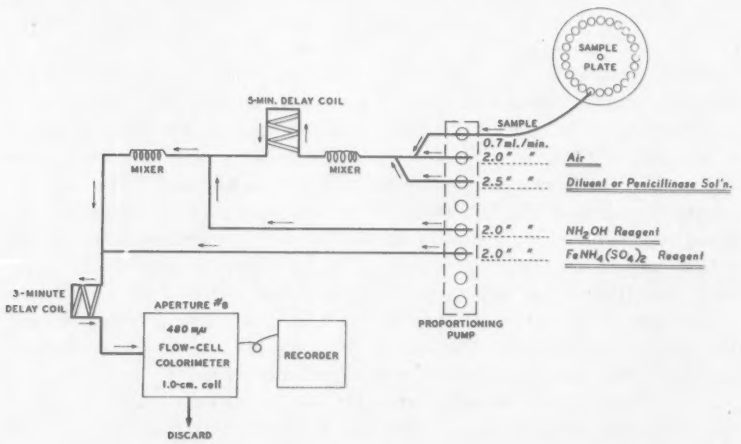

FIGURE 5. Flow diagram of penicillin hydroxylamine method.

A typical standard curve using aqueous penicillin standards is seen in FIGURE 6. Experience has shown that the standard curve is reproducible from day to day within an over-all error of 1 per cent or less, and no curvature or lack of parallel response of unknowns, blanks, or standards has been encountered. The method was further evaluated by a

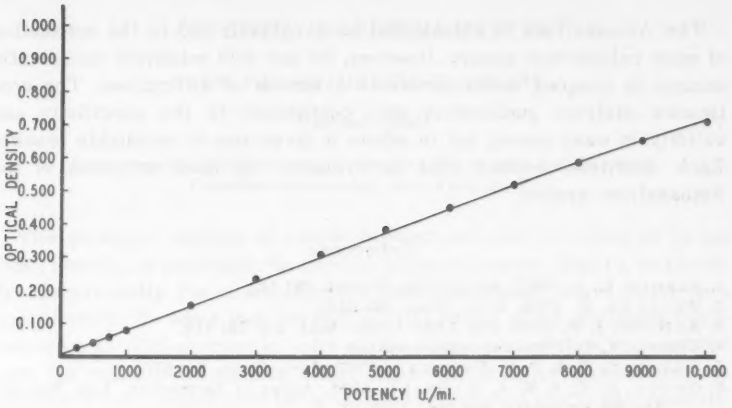

FIGURE 6. Aqueous penicillin standardization.

crossover experiment with a microbiological diffusion assay[6] on 18 various fermentation samples. The data, set forth in TABLE 1, demonstrate the ability of the method to measure penicillin potency.

TABLE 1

COMPARISON OF AUTOANALYZER-HYDROXYLAMINE METHOD AND MICROBIOLOGICAL-DIFFUSION METHOD FOR THE DETERMINATION OF PENICILLIN IN FERMENTATION BROTHS USING THE SIX-POINT DESIGN

Broth sample	Penicillin potency, u./ml.	
	Autoanalyzer	*Microbiological Diffusion*
1	3437	3336
2	2056	2270
3	3327	3524
4	2143	2266
5	3841	4355
6	4144	4651
7	3338	3856
8	1090	1090
9	3053	3285
10	1481	1634
11	3948	4298
12	4447	4978
13	4487	4582
14	2626	2902
15	4405	4608
16	4533	4473
17	4113	4179
18	4725	5193

The Autoanalyzer is established as a valuable aid in the automation of many colorimetric assays. However, its use with relatively nonspecific assays in complex media generates a number of difficulties. The continuous dialysis purification step contributed to the specificity and validity in many cases, but in others it gives rise to unreliable results. Each analytical method must be evaluated by itself in terms of the Autoanalyzer system.

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*DIVISION OF BIOCHEMISTRY**

ON THE NATURE OF CANCER**

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The principal problem of cancer research is still the same as in the past, namely, to determine the precise nature of cancer: that is, to identify unequivocally the characteristics that distinguish cancer cells from normal cells. To admit that the fundamental nature of the malignant transformation is still obscure in spite of the large mass of data accumulated over the years of research is not to disparage these researches. Such an admission only emphasizes the need for venturesome stochastic generalization in cancer research.

I propose to make such generalizations. I shall develop my hypotheses from commonplace self-evident definitions of cancer reinterpreted more restrictedly in accordance with the objective of eventually pinpointing the metabolic defects that must characterize cancer. The experiments I describe serve only to test my hypothetical conclusions. I shall dwell only upon those properties of cancer cells that may serve to distinguish them from normal cells, and shall postpone to another day considerations of any particular associated vulnerabilities which might suggest therapeutic measures.

The axiom that cancers are composed of cells that grow without restraint or control focuses attention on the two definitive features of malignant behavior, avid growth and autonomy. The growth property may be emphasized by specifying more narrowly that the most characteristic aberration of cancer cells is that they grow without cessation: cancer cells must either grow or die.

As regards growth and growth controls: in our search for experimental simplicity it occurred to me and my associates that cell culture, which has proved so fruitful in morphologic studies, could be even more useful in metabolic studies, and might serve as an ideal system to test our hypotheses. We modified the conventional methods of tissue culture somewhat in order to obtain certain advantages and conveniences.[1] FIGURE 1 is a diagram of the apparatus that evolved. The cytogenerator differs in

*The Division of Psychology held a meeting on March 16, 1959 at which Solomon E. Asch of the Institute of Advanced Study, Princeton University, Princeton, N. J., presented a paper entitled "Perceptual Conditions of Association." This paper has not been received by the Academy.

**This paper, illustrated with slides, was presented at a meeting of the Division on March 19, 1959. The work described in this article was supported by grants from the Damon Runyon Memorial Fund, New York, N. Y., and from the Public Health Service, Bethesda, Md.

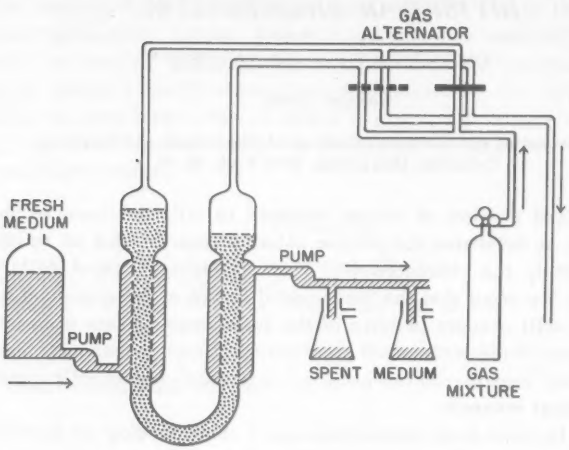

FIGURE 1. Schema of cytogenerator system.

certain respects from the familiar chemostat; all of the cells produced are retained within the growth chamber in suspension unless they are removed manually, whereas the chemostat maintains a constant population by discharging cells as they are produced. Fresh nutrients are delivered to the growth chamber by perfusion through a septum, and spent medium is removed in similar fashion. The composition of the gas phase is controlled and variable. We have been able to grow or maintain, as desired, a number of different pure cell strains of either normal or malignant origin continuously for several weeks, without transfer or reinoculation under controlled conditions convenient for metabolic studies of some variety. Large masses of cells, 10 to 15 gm., were readily obtained in a short time in one 500-ml. cytogenerator flask. That this culture system is an improvement over conventional methods is attested by the fact that we have been able in this arrangement to grow Ehrlich ascites outside the animal body for the first time. Ehrlich ascites is a most convenient form of cancer for biochemical studies but, hitherto, investigators have had to grow them in the living animal and accept the many experimental limitations so imposed.

FIGURE 2 illustrates the reproduction of cells in the apparatus. In this instance the cytogenerator carries a mother culture of fibroblasts from which experimental subcultures are seeded. The consistency of reproduction rates is typical.

The walls of the U tube are of fritted glass with pore size just small enough to retain the cells, but large enough to permit free transfer of nutrient fluids. Fortunately, rather coarse, sintered glass candles are adequate for practically all animal cells. Smaller microbial cells would

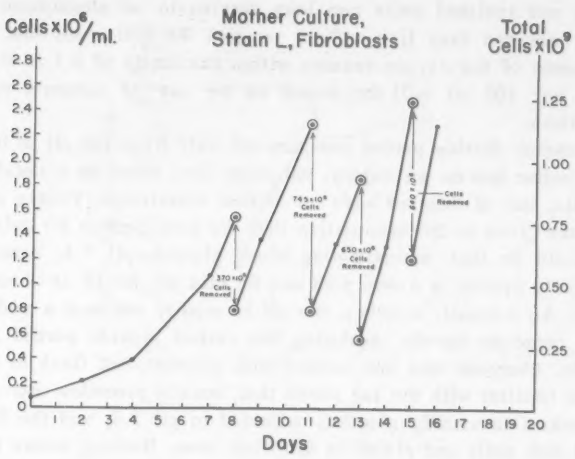

FIGURE 2. Growth and regrowth in the cytogenerator.

not be retained in these chambers. However, we have used semipermeable membranes successfully instead of fritted glass for culturing bacteria that usually do not require high molecular weight dietary components as do animal cells. The requirement for high molecular weight components by animal cells, usually as serum proteins, virtually dictates the method of agitation and of gas transfer.

Note the two fritted glass candles, used not only to provide greater transfer area, but also to facilitate reversal of the nutrient stream in those instances where overly luxuriant growth might clog the efferent candle. The variation in diameter of the tubular columns creates a moderate turbulence as the fluid level rises and falls. Accurate matched variable pumps are used to keep the outflow exactly equal to the inflow in order to maintain the volume constant. Alternate gas pressure pulses provide sufficient turbulence to hold the cells in suspension while equilibrating the nutrient solutions with the desired gases. The amplitude of the pulse and its reversal are controlled by the double-pole, double-throw reversing switch.

The partial pressures of oxygen and carbon dioxide in the gas phase influence cellular events in various ways. Almost all terrestrial animals are accustomed to sea-level air that contains about 21 per cent oxygen: that is, they breathe 21 per cent oxygen, but the tension of this gas at the reacting cellular loci is, of course, much less; probably no more than 0.5 cc. per hundred grams of tissue. The intact animal uses hemoglobin for oxygen transport but, in this device as well as in the Warburg flask, we must rely on Henry's law. The solubility of oxygen in the nutrient fluid under 21 per cent oxygen is about 0.5 per cent per 100 ml. Neither

animals nor isolated cells can long survive in an atmosphere of pure oxygen, nor can they live without oxygen. We anticipate that delicate adjustments of the oxygen tension within the limits of 0.1 to 0.5 cc. of oxygen per 100 ml. will be found to be one of nature's regulatory mechanisms.

The carbon dioxide partial pressure not only fixes the *p*H of the bicarbonate buffer system we employ, but might also serve as a metabolite for the cells, but of this we have no factual knowledge. Tissue culturists were once given to the supposition that the best medium for cell cultivation would be that approximating blood plasma, *p*H 7.4. However, the bicarbonate system is a very poor one for this *p*H; its *p*K is about 6.3 and not 7.4. As a result, however, the *p*H is readily set over a wide experimental range by merely regulating the carbon dioxide partial pressure.

Today, everyone who has worked with conventional flask or tube cultures is familiar with the lag phase that usually precedes active growth. The medium is usually carefully adjusted to *p*H 7.4, and the flasks are seeded with cells and placed in the warm room. Nothing seems to happen for a time, but eventually growth commences. We have found, however, that one can abolish the lag period or, on the other hand, impose one for any desired length of time by simply setting the selected *p*H with CO_2. The fact is that cellular replication takes place only in a relatively acid medium, that is, close to *p*H 7.0. We have not found any cell line that can grow at *p*H 7.6. At the more alkaline *p*H normal cells (in most of our experiments we use Earle's L strain mouse fibroblasts) survive without change in cell number for long periods. These nonreplicating cells respire, consume glucose, and produce lactic acid, which, if it were permitted to accumulate, would soon bring about the acid state necessary for replication. A culture of normal cells can be converted to a maintenance culture simply by raising the *p*H. This convenient property permits us to study replication and maintenance separately for the first time, for in the well-ordered society of normal cell culture there appears to be no death.

However, we have ventured the proposition that cancer cells must either multiply or perish: that is, that they cannot undergo a maintenance status as can normal cells. FIGURE 3 shows that this is indeed the case when we subject Ehrlich ascites cells to *p*H 7.6. Tumor cells cannot enter a maintenance phase. The seemingly nongrowing or static tumor must be one in which a high cell death rate balances the high proliferation rate.

When normal cells enter a maintenance phase their metabolic behavior changes also; they utilize the dietary metabolites in a different way. The medium we employed is a complex one, containing an assortment of vitamins, minerals, amino acids, serum protein, and glucose, the detailed requirements having been worked out by Earle[2] and by Eagle.[3] It is known as a defined medium, possibly because it contains only one undefined component, horse serum. We do not know, of course, whether such a medium can support all cells, nor whether the components are present in

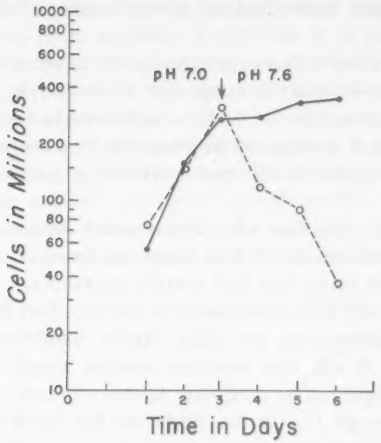

FIGURE 3. The influence of pH on cell cultures. Key: o———o———o is a normal cell line, strain L, clone 929, fibroblasts; o-----o-----o is a cancer cell line, Ehrlich ascites.

optimum relative proportions for maximum growth. We have made observations on three of the components thus far: serum, glutamine, and glucose.

The culture of animal cells differs from that of bacteria in that the former requires the inclusion of protein in the medium; the equivalence in amino acids will not suffice. This protein is ordinarily supplied in the form of serum, and we are still uncertain about the precise contribution of the serum; that is, whether it contributes only protein or also supplies protein-bound carbohydrates or lipids that might be essential for culture. Not only is the serum indispensible, its concentration also influences the replication rate proportionately. Amino acids cannot replace serum, but neither can amino acid-free serum alone support replication. TABLE 1 demonstrates some representative balance data from studies with fibro-

TABLE 1

NITROGEN BALANCE OF FIBROBLAST CULTURES

			Cell N		*Medium N*		*−Δ Prot N*	*Glucose equiv.*
Exp.	*GT*	*Time hrs.*	*Start mg.*	*End mg.*	*−Δ AA mg.*	*−Δ Prot. mg.*	*New N*	*mg./day/mg. cells*
1	61	336	0.29	8.38	4.6	87.4	10.8	0.57
2	118	120	9.1	19.6	6.2	114	10.8	0.43
3	∞	216	9.6	9.5	1.2	0.7	–	–

blasts. These figures were obtained by the analysis of the afferent and the efferent media.

First, note that some amino acid nitrogen is taken up from the medium, but hardly in sufficient quantity to account for the newly produced protein. The amino acid data are uncertain by a wide margin because our analytical methods could not distinguish between the free amino acids originally provided and the amino acids that could have arisen from the serum protein.

Second, however, note that very considerable amounts of protein have disappeared from the medium; in fact, about ten times as much disappeared as can be accounted for by new cell protein synthesis.

Third, however, and most interesting, is the fact that the nonreplicating, presumably nonsynthesizing yet fully viable maintenance culture consumed no protein at all. One wonders whether simply removing protein from the medium might not be sufficient to throw it into maintenance.

We have been taught that amino acids are the building blocks for protein synthesis, and that animals can subsist, even grow, on a protein-free diet, but the observation that cell cultures require protein and, most probably, do not degrade this dietary protein all of the way to amino acids suggests that either the capacity to synthesize proteins from amino acids is not common to all cells, or that our cell culture systems are sadly deficient in yet unknown but important factors.

Whether or not an appropriate selection of peptides would serve as well as whole serum proteins is still to be determined. The fact that so much protein is expended without incorporation suggests that it might well serve another purpose, that is, that the protein is not only a source of energy required for replicative synthesis, but is also readily available for the purpose. If we think of the protein as a carbohydrate chain, that is, as a donor of gluconeogenic amino acids of which it is composed in large part, and make a rough calculation of the glucose equivalence of the amount of protein expended, we find that it approximates about 0.5 mg. of glucose per day per mg.; an appreciable quantity, as we shall see later when we consider the glucose consumption.

In the same vein we face still another intriguing puzzle in the essentiality of glutamine – not glutamic acid – for cell culture. Glutamine is a high-energy compound. Its role will not be completely elucidated until we can draw up an energy balance sheet for cellular replication. The glutamine added to the medium soon disappears; only glutamic acid can be found in the efferent stream. Quantitative experiments with N^{15} glutamine show that the fibroblasts themselves synthesize about one half the glutamine they require, an uncomfortable situation for one who likes absolutes; the cells should produce all of the glutamine they need or none.

It is often said that carbohydrate is the main source of energy for living cells. Some of us doubt this but, regardless of our uncertainty, we find it

necessary to supply large amounts of glucose to the medium. Cells in culture do consume large amounts of glucose. It is not always realized that a well-behaved conventional culture can *exhaust* its glucose in about six hours. Indeed, experiments with the more usual flask or tube cultures often consist in the observation of cells while they run the gamut of feasting to fasting. However, when the perfusion system is employed in such a manner that the efferent glucose never falls below one half the afferent concentration, cell numbers increase logarithmically, as shown in FIGURE 4, *p*H 7, of course. Ascites cells, for example, consume glucose so avidly that we often fail to keep pace in spite of our best efforts.

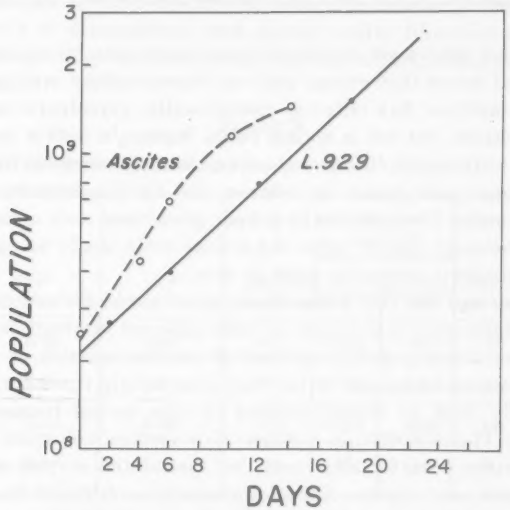

FIGURE 4. Reproduction rates in the cytogenerator.

TABLE 2 shows the magnitude of the glucose consumption under different conditions. Note that the glucose consumption is only about 30 per cent less in the nonreplicating maintenance system. A great deal of potential energy is locked up in that much glucose, but these cells do not get much of it. About 80 per cent of the glucose is converted to lactic acid, except, curiously enough, in the nonreplicating system, which would not be expected to need as much energy. Glycolysis, then, is not intrinsic to the cell but, rather, a manifestation of growth. The maintenance status is that in which protein is not used, whether for *de novo* synthesis or for energy; carbohydrates appear to suffice at that time.

Ascites cells produce even more lactic acid than do normal growing cells, about 50 per cent more, in fact, than can be accounted for by their consumption of glucose. This fact illustrates and supports the Warburg

TABLE 2

GLUCOSE CONSUMPTION IN CULTURES

Exp.	pH	*GT*	*Gl. used mg./day/mg.*	*LA mg./day/mg.*	*LA/gl. × 100 per cent*
L,929	7.2	61	1.16	0.94	81
L,929	7.2	118	1.01	0.75	74
L,929	7.6	∞	0.79	0.02	2.5
Ascites	7.2	50	2.20	3.30	150

hypothesis that tumors are characteristically and distinctively glycolytic.[4] Warburg, well aware that normal cells in tissue culture are also strongly glycolytic, qualified this criterion operationally; glycolysis *exceeds* respiration in cancer, but not in normal cells. Warburg's further qualification is very much to the point; *"Wenn wir sagen, dass die normalen Körperzellen nur atmen und nicht gären, so müssen wir die Einschränkung machen, dass die normalen Körperzellen im Körper gewachsen sein müssen."** The distinction between cancer cells and normal cells would be even sharper if the measurements were also made *in vivo*.

Some years ago the late Jesse Greenstein[5] made the now familiar and important observation that tumors of quite different etiology and histogenesis all have a nearly uniform pattern of metabolism; that no matter how or from what tissues tumors arise, they more nearly resemble each other biochemically than do normal tissues or than normal tissues resemble each other. The distribution pattern of a random selection of enzyme activities is the same for all tumors, but the related enzyme activities of normal organs vary widely. Normal tissues have different functional capacities, but tumors appear to have but one overriding function, growth.

Cell culture manifests growth in the complete absence of host influences. However, the restrictive circumstances of orthodox cell culture are not conducive to the exercise of diverse cellular powers other than growth. Indeed, Lieberman and Ove[6] have shown that cell cultures originally seeded from different organs cannot be distinguished from each other enzymatically. Even a malignant line, HeLa, has the same enzyme pattern as do several different normal cell cultures. Cells in culture at *p*H 7 have one common overriding function: growth.

However, let us reexamine our *in vitro* experiments. The idea of simulating *in vivo* conditions was implicit in the beginnings of cell culture. We were satisfied too easily, however; we stopped too soon. We allowed

*"When we say that normal cells only breathe and do not ferment, we must qualify this statement by adding that normal cells must have been grown in the body." (Editor's translation.)

some of our superficial successes to blind us to the fact that we had not simulated *in vivo* conditions very well. We were tempted to make biological conclusions and comparisons from highly artificial and perhaps wholly unnatural circumstances.

We now submit that cancer cells cannot stop growing because they lack the capacity to respond to host regulatory mechanism. We must now test this hypothesis by introducing the appropriate hormones, one by one, into our culture system; we must specify not only the hormones involved, but also the key metabolic reactions over which they are presumed to reside. To begin with, we postulate a master hormone to which normal cells can and do respond, but to which cancer cells must prove refractory by virtue of their intrinsic genetic defect. We nominate insulin to this important role. TABLE 3 demonstrates that normal cells, fibroblasts, do indeed react to insulin. Ascites cells do not react at all according to the parameters measured.

TABLE 3

EFFECT OF INSULIN ON GLUCOSE UTILIZATION

Exp.	GI. mg./day/mg.	LA mq./day/mq.	LA/GL. per cent	Radioactivity cts./mg./min. Aff.	Radioactivity cts./mg./min. Eff.	Eff./Aff. per cent	GI. mt.
Control	1.16	0.94	81	6160	6050	98	0.22
Control	1.10	0.87	79	5770	5640	98	0.23
Plus ins.	0.33	0.08	37	2550	1680	66	0.25
Plus ins.	0.36	0.08	38	2560	1545	60	0.28

Insulin invokes gluconeogenesis in fibroblast cultures. Prevailing concepts of insulin action do not predict such an effect. The amount of glucose newly formed in these experiments was probably much higher than the ratios indicate because a high perfusion rate was used in these experiments in the expectancy that insulin would increase the consumption of glucose; therefore the newly-formed nonradioactive glucose was overly diluted by radioactive medium glucose. Quite to the contrary, insulin reduces the consumption of glucose by these cells, seemingly by about 80 per cent, but more rigidly by about 66 per cent if we correct for gluconeogenesis. Note further that the conversion of glucose to lactic acid was similarly reduced. One could now expect, however, that lactic acid production would be completely abolished if the experiment could be conducted under ideal conditions, but it is precisely these ideal conditions we have yet to define. We hope to realize these conditions by further simulation of the host environment.

However, a few interesting propositions emerge even under these less-than-ideal conditions. For example, if one deducts the lactic acid formed from the glucose consumed, one arrives at the same number, whether or not insulin is offered to the cells. This number, about 0.25 mg. of glucose per day per mg. of cells, includes both the amount of glucose completely degraded to CO_2 (which we have not yet been able to measure in these experiments) and the glucose carbon incorporated into cell structures. TABLE 4 contains representative data not only illustrating the magnitude of the glucose contribution to protein, fat, ribosenucleic acid (RNA), and desoxyribosenucleic acid (DNA), but also demonstrating that insulin does not influence the structural incorporation of glucose, and that even non-replicating cells incorporate about the same amount of glucose. These cellular components and, surprisingly, even DNA, are in dynamic equilibrium with the environment and are not affected by either reproductive status or by insulin.

TABLE 4

CONTRIBUTION OF GLUCOSE TO CELLULAR STRUCTURES

Exp.	*Gens.*	*Hrs.*	*Fat* percentages*	*Prot.* percentages*	*RNA* percentages*	*DNA* percentages*
1	5.5	336	4.7	9.7	8.9	11.0
2	6.8	336	4.7	9.6	10.6	14.0
3	0	216	5.5	8.7	6.6	4.4
4†	6.2	336	4.0	11.8	10.8	11.8
5†	7.0	336	4.0	11.4	13.9	17.0

* Ratio of specific activity to specific activity of medium glucose.
† Plus insulin.

The aberrant use of glucose by the hormone free cells, the exceptional effects produced by insulin, and the continued eccentricity of ascites cells treated with insulin appear to center about pyruvic acid, an important branch point in carbohydrate metabolism. Pyruvate can be reduced to lactic acid, or it can enter the tricarboxylic acid (TCA) cycle. Pyruvate can enter the TCA cycle in either of two ways: it can be decarboxylated to yield acetyl CoA, whence it enters the TCA cycle for energy-yielding purposes; or it can condense with CO_2 to form dicarboxylic acids, yielding a net increase in TCA cycle mass. The relative proportions of pyruvate entering the TCA cycle by these two different pathways can be estimated by injecting precursors of pyruvate C^{14} into intact animals and subsequently measuring the relative radioactivities of the individual carbon atoms of glutamic acid isolated from the tissues or organs in question.[6]

It can be reasoned from FIGURE 5 that: (1) carbon 5 of glutamic acid will be labeled if pyruvate-2C^{14} enters the cycle by decarboxylation to acetyl-1C^{14}; (2) carbons 2 and 3 will be labeled when pyruvate is converted to a dicarboxylic acid by fixation of CO_2; and (3) carbon 4 will be labeled if and when dicarboxylic acids of the TCA cycle are converted to acetyl CoA.

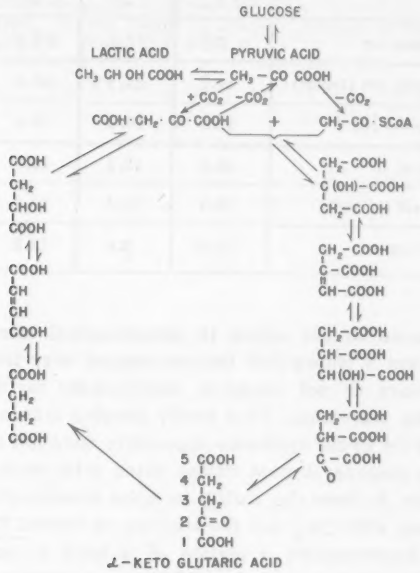

FIGURE 5. The two modes of entry of pyruvic acid into the TCA cycle.

TABLE 5 shows the results of an experiment designed to compare the effects of feeding and starving on the metabolic pathways of pyruvate. These labeling patterns show that in the liver of the starved animal carbohydrate enters the TCA cycle primarily as a dicarboxylic acid rather than as acetyl CoA. The principal energy source in the liver of the fasted animal is probably fat, the carbohydrate being conserved for TCA cycle synthesis. In the rat given glucose, however, an appreciable portion of the pyruvate entering the TCA cycle does so by decarboxylation to a two-carbon fragment. The small labeling of carbon 4 shows that only a negligible proportion of TCA dicarboxylic acids is decarboxylated to two-carbon fragments in the livers of either starved or fed rats. The liver, a normal organ, readjusts its metabolic pathways to meet environmental circumstances. Presumably, this readjustment is a feature of hormonal regulation.

TABLE 5

RELATIVE RADIOACTIVITY OF INDIVIDUAL CARBON ATOMS OF GLUTAMIC ACID FROM RAT LIVERS AND MURPHY-STURM LYMPHOSARCOMAS

	Experiment	Carbon number				
		1	2	3	4	5
1	Liver, starved rat	7.7	27.5	56.3	0.8	3.1
2	Liver, starved rat (tumor)	14.2	25.3	54.2	0	3.9
3	Tumor, starved rat	19.8	14.1	33.9	9.0	21.0
4	Liver, fed rat	15.3	18.1	40.7	1.1	20.3
5	Liver, fed rat (tumor)	20.5	12.3	19.2	2.0	40.4
6	Tumor, fed rat	23.9	8.6	18.8	7.7	40.2

However, tumors do not adjust to environmental circumstances. The labeling of carbon 5 shows that tumors respond very little, if at all, to starvation; tumors do not conserve carbohydrate for dicarboxylic acid synthesis during starvation. This avidly growing tissue that has an exalted level of TCA cycle synthesis apparently does not depend on carbohydrate for this purpose; it must utilize amino acids such as glutamic and aspartic. Tumors do have the ability to make dicarboxylic acids by condensing pyruvate with CO_2, but the labeling of carbon 4 shows that they improvidently decarboxylate a portion of it back to acetyl CoA. They consume the furnace along with the fuel! One can observe the aimlessness of tumor carbohydrate metabolism even in the intact animal. Cancers, unlike normal cells, behave alike, both inside and outside the body.

These experiments contribute to the definition of cancer as the resultant of mutational processes that give rise to new races of cells that do not have the capacity to mature or to differentiate completely in response to host regulatory mechanisms.[7] The data demonstrate that, even in their fixed immaturity, cancer cells exhibit functional aberrations that derive from their deficient status. The emphasis on glycolysis is a consequence of their inability to respond to host regulatory mechanisms. The cell culture experiments suggest that insulin might well be one of the critical regulators in this respect.

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DIVISION OF ENGINEERING

IRON ORE REDUCTION *

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Inventors, governments, and companies are working vigorously, and at great expense, to develop new processes for the extraction of iron from its ores. These activities have been increasing in scale and cost for several decades until they have reached the point where several of these new processes have been placed in commercial operation in selected localities.

At the same time, however, the blast furnace is being used with great success in many lands for the economical production of iron on a very large scale. What is the incentive that leads so many inventors and their sponsors to continue working in this field? An answer to this question can be proposed from a consideration of the history of ironmaking.

In the earliest times meteoric iron was used in jewelry and talismans. The charcoal reduction process came into being about 1000 B.C., probably in Asia Minor. The process required the use of relatively high grade ore and abundant supplies of wood charcoal. Production centers arose in many parts of Europe and Asia, and iron soon became too cheap for conspicuous or magical use. Iron tools and weapons appeared, and the Iron Age began in the Eastern Hemisphere.

The Assyrians were the first people to understand the military potentialities of the new metal. Their king, Sargon II, equipped his soldiers with iron weapons about 700 B.C. and developed new tactics and military engines for the Iron Age. Their neighbors, still fighting with bronze spears and leather shields, were hopelessly outclassed, and the men of Nineveh soon ruled their known world. Having achieved great technical superiority over all comers, they behaved with such ferocious cruelty that they quickly came to grief. Weakened by internal decay and infiltrated by enemy agents, the Assyrian nation was totally destroyed by an international coalition only a century after the general arming of their forces with iron apparatus.

At about the time of the destruction of Nineveh (600 B.C.), a great iron center arose in the region of Austria (then called Noricum). Here the iron ore was low in sulfur and phosphorous and high in manganese, a most fortunate combination that enabled the German smiths to produce a relatively hard steel for use in making swords and knives. This Noricum steel was widely traded throughout Europe and, we may assume,

*This paper was presented at a meeting of the Division on March 20, 1959.

came to the attention of the governments in Eastern Europe. In about 350 B.C. Philip of Macedonia began his preparations for the conquest of Asia. Like Sargon, he armed his troops with the best war materials of the time and trained them in the technicalities of his military innovations, including the use of armored, disciplined cavalry. Commanded by Philip's son, Alexander, the Europeans penetrated deep into the heart of Asia, meeting very little resistance along the way. Turned back at the borders of India, where they encountered weapons as metallurgically advanced as their own, the Europeans retreated slowly toward their homeland.

Asiatic metallurgy developed rapidly; ironmaking became a specialty in India, while steel-forging and weapon-forming became a great industry in the Near East. The famous swords of Damascus were made in that city from iron billets imported from India.

During the Fourth Century A.D. the Asiatics set out upon a systematic invasion of the European continent; in due time the Huns established a great state throughout most of Northern Europe. Stopped only in Western France (about 450 A.D.), the Hunnish tide disintegrated upon the death of their last great king, Attila.

During the Eighth Century the Asiatics struck again at Europe. Backed by their rapidly advancing steel technology (carburizing, quenching, and heat-treating were now well known in Asia), the Arabs and their allies occupied Spain and most of France. Additional large-scale Asiatic penetrations by the Mongols of Genghis Khan deep into Europe occurred during the Thirteenth Century. The last major incursion into the body of Europe from Asia was carried out by the Ottoman Turks, whose rush was finally brought to a halt in 1683 at their unsuccessful siege of Vienna.

During these times of repeated massive onslaught against Europe by the militarists of Asia, the charcoal reduction process for making iron was widely practiced throughout the Old World. Toward the close of this period, however, the deforestation of the inhabited parts of Europe and Asia had advanced to the point where it was becoming increasingly difficult to obtain enough wood charcoal to meet the growing demand for iron. During the Sixteenth and Seventeenth centuries, European iron men experimented with other forms of carbon. These men mixed wood charcoal with various kinds of coal, coke, and lignite in an attempt to stretch the diminishing charcoal supply and to find a substitute. Finally the great technical breakthrough occurred in 1727, when Abraham Darby, in England, succeeded in using a particular kind of coke for the complete replacement of charcoal in the iron furnace.

British ironmasters quickly took up Darby's invention and, blessed by fortune with large deposits of coking coal, they built up the greatest steel industry known up to that time. Certain fortunate regions on the Continent were able to follow the English lead, as did the Americans when their forests were nearly gone. However, in India the tables were turned decisively. Not endowed with comparable deposits of coking coal,

India's steel production was limited by its forest growth, and the people of India were therefore unable to expand their output of weapons after Darby's great discovery.

In the second half of the Eighteenth Century Europe, for the first time in more than one thousand years, was able to carry out a large-scale invasion of Asia. In the form of the British East India Company, the English soon took control of one of the most advanced civilizations on the larger continent. This historic shift in the balance of power was wistfully recalled by Jawaharlal Nehru in his opening remarks to the meeting of the Board of Governors of the World Bank in India in October 1958. Nehru remarked that when Europeans appeared in India during the Sixteenth and Seventeenth centuries they came to purchase the superior manufactured goods of India, which were in great demand in Europe. The Indian leader then noted that today the level of technology and industry in India is far lower than that of Europe, and he viewed this strange shift in technical leadership and political power somewhat enigmatically. At least some part of this great transformation may well be attributed to the change in the relative position of Indian steel technology with respect to that of Europe that occurred during the Eighteenth and Nineteenth centuries.

Since the great days of the East India Company, most of the world's iron production has been carried out in the coke blast furnace. Great nations have developed around those regions sufficiently fortunate to possess large supplies of coking coal. The present world map clearly shows that the localities that today have a high standard of living are principally those liberally supplied with coke and coal. However, the poverty of many areas in the world, if one accepts for a moment this ferrous hypothesis, is not due to a lack of iron ore. India, China, South America, and many parts of Africa are liberally supplied with high grade ore. What they lack is a good supply of a suitable reducing agent. It is this deficiency of reducing agents that is supplying the incentive for our modern efforts to develop new ironmaking processes. Other motives for this great effort in research and development may be connected with shortages of capital and the exhaustion of high-purity ore deposits, but the great impetus comes from the mounting demand for iron in the "cokeless" areas of the world.

The actual chemical attack on iron ore is made, of course, by carbon monoxide or hydrogen, or by mixtures of the two, and these gases can be prepared from such substances as coal, lignite, coke, natural gas, and petroleum. In general, each of the new processes starts with some of these materials and uses them to make the reducing gas and to supply heat. In appraising them, we look first at the net energy consumed per unit of iron produced. On this point the literature is confusing and contradictory. At the Kellogg Company we have made a detailed study of

these reports and have calculated the energy demands as accurately as possible. Not surprisingly, the First Law of Thermodynamics still holds, and processes doing the same work use about the same total energy, certain published claims notwithstanding. After allowing proper credit for the heat content of by-products, and basing all operations on the same ore, we find that most of the new processes require almost exactly the same net energy input as does the blast furnace. Some designs using electricity appear to require less energy than others, but this advantage is lost if we calculate the fuel required to generate this electricity in a typical steam-generating station. The net fuel load, be it coal, lignite, methane, or petroleum, is about 15,000,000 B.T.U. per ton of iron. Therefore there will be little variation in true operating costs in well-engineered plants, the advantage in any given locality going to the processes that use the cheapest local source of reductant and heat.

The little information available on capital costs suggests that the new processes will be cheaper than that of the blast furnace, but this inference must be viewed with caution. The new processes have been actually proved in amounts no larger than 200 tons per day, while modern blast furnaces produce more than 2000 tons per day. Capital cost estimates for large-scale units using the new processes are therefore extrapolations exceeding a tenfold increase: a dangerous extension in dealing with processes in which flow distribution and mixing are very important.

It is true that the United States steel industry seems interested in these new processes, doubtless in the hope that they will lead to greatly reduced capital cost. The needs of the country's industry are particularly acute because of the inadequate depreciation reserves accumulated by most of the companies. However, a number of years must pass before any of the new processes are perfected in the 2000 tons/day size, and only then will they become really interesting to the larger steel companies. Meanwhile, the capital available during the next few years will usually be better spent on ore purification and pretreatment.

In this connection, it is to be noted that some processes can handle relatively impure ores, but with a proportionate increase in energy consumption and operating cost. I believe, moreover, that this is generally not the optimum design for a process unit. The steel industry is rapidly discovering the wisdom of following the chemical industry's familiar practice of purifying the raw materials before charging them to the main reactor.

Finally, in appraising any new process, the question of operability must be carefully studied. The ore-reduction processes work in a very difficult region of chemical and physical factors. Temperatures are high and environments are corrosive. While the reactants must be intimately mixed, their solid or pasty consistency makes flow distribution poor and mixing irregular. Plant size must therefore be extrapolated with great

caution and only after prolonged, successful operation has been achieved in the initial units.

The iron ore-reduction methods can be grouped into five major categories: (1) moving bed; (2) rotary kiln; (3) electric furnace; (4) fluidized bed; and (5) fixed bed.

The principal moving-bed unit is, of course, the blast furnace, which has been built in sizes up to about 3000 tons per day at an investment cost of $80 to $100 per ton-year capacity. The Wiberg process is a moving-bed method that does not require metallurgical coke and that produces sponge iron. It has operated commercially in Sweden for many years with a capacity of about 100 tons per day.

Many rotary kiln processes are undergoing development at the present time. The Krupp-Renn process has been placed in commercial operation with a capacity of about 200 tons per day. The best known of the electric furnace processes, which are economic only where electricity is very cheap, is the Tysland-Hole process. This has been extensively used in Norway in capacities of about 100 tons per day. It does not require metallurgical coke, and must certainly be considered for commercial application where electricity is cheap and where the smaller-sized units are commercially attractive.

The fluidized bed units, which have received a great deal of publicity, are all in the development stage and should be so considered for the immediate future. A 50-ton-per-day plant of one of the fluidized processes should be in operation soon. Among the fixed-bed processes I may mention that of Höganäs and that of HyL. The former is suited to small-scale production of high-purity iron, of the type required for powder metallurgy. The HyL process is now operating commercially in Monterrey, Mexico at a capacity of 200 tons per day. The resultant sponge iron is converted to steel in electric furnaces. A larger plant (500 tons per day) embodying the HyL process is now under construction at Monterrey.

In addition to the processes described above as having had some commercial application, there are many other novel schemes and ore-reduction proposals that are in various stages of research and development. At present, the blast furnace is the only universal process, thoroughly demonstrated in large-scale applications. In the course of time we expect that some of the new processes will be installed in regions deficient in metallurgical coke. We expect that the blast furnace will slowly lose ground unless, of course, ways can be found for converting low-grade coal into metallurgical coke. Generally, however, we anticipate that the new processes will eventually find wide application and will be developed to large capacities in regions supplied with natural gas, petroleum, lignite, and other noncoke reducing agents.

In attempting to predict the political consequences, if any, that may follow from the widespread use of the new iron ore-reduction processes we realize that a daring – even foolhardy – proposition has been advanced in this paper. It has been suggested that the relative development of iron and steel technology plays a powerful part in determining large-scale political changes. Of course, this theory is a gross oversimplification of the complicated historical process. There are far more things in life than austenite and martensite, but it is sometimes possible to obtain an understanding of the major elements in a complex process by means of highly simplified models. This, at least, has been the experience of engineers in their general attack upon physical problems.

This ferrous hypothesis is offered, therefore, as a crude model (highly "linearized," as the mathematicians would say) which might have some utility for purposes of prediction. In attempting to look into the future, we notice that almost all of the backward countries that are now struggling to raise their standard of living place the utmost importance on increasing their own production of iron and steel. The Indian program for this expansion was put in action as soon as the Nehru government took control. Perhaps the most noteworthy supporters of our iron model are to be found in China. The new Chinese government is making frantic and incredible efforts to increase iron production. As we have learned from recent reports, thousands of small primitive blast furnaces are being built and operated with extravagant amounts of hand labor in China today. We cannot say whether the Chinese are more concerned with establishing a ferrous equilibrium with the United States than they are with the Union of Soviet Socialist Republics, but it seems clear that they consider the possession of a large steel-making capacity a vital step in their plans for restoring their national greatness.

The widespread commercial application of the new iron processes in the cokeless areas of the world could be very beneficial. While this paper has dealt principally with the use of iron in warfare, there is no intention of overlooking the enormous value of iron and steel in time of peace. Properly used, therefore, the new processes can raise the standard of living of a large part of the world's population and can bring about a more stable distribution of political power. The appearance of a new Sargon or Alexander to resume the course of conquest would be a sad blow to humanity. The prevention of such an occurrence is a major task for coming generations.

DIVISION OF MYCOLOGY

THE CENTRIOLE AS THE PRIMARY RADIATION TARGET IN THE RESTING CELL*

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Introduction

The direct correspondence between the degree of ploidy and the number of centrioles in the centrosome (Yuasa and Lindegren, in press) has shown that the centrosomes in haploids contain a single centriole; the centrosomes in diploids contain two; triploids, three; and tetraploids, four.

The multitarget theory of radiation inactivation implies a morphologic target. Lucke and Sarachek (1953) found that the "average survival curves for haploid, diploid, triploid, and tetraploid (after X-ray treatment) give extrapolates of 1, 2, 3, and 4, respectively." Morphologic targets involved in the radiation inactivation of yeast cells that can be related to the mathematical model have hitherto evaded detection. The centrosome cannot be the primary target since there is only one centrosome in all cells regardless of the degree of ploidy, and the centrosome is a single homogeneous morphologic unit. The fact that the number of centrioles increases in an integral way with the degree of ploidy offers a direct means for determining whether the centriole is a primary target of radiation (X-ray) inactivation. The recognition of the relation of the centrioles to ploidy by Yuasa and Lindegren makes it possible to test the theory since, on the multitarget theory, the majority of the survivors of radiation, occurring during the straight-line portion of the survival curve (FIGURE 1), should contain only one target, the other target or targets having been lost.

Materials and Methods

Cells of diploid culture 11294 grown for 3 weeks on glucose-nutrient-agar were harvested, washed 3 times in M/15 KH_2PO_4, and resuspended in M/15 KH_2PO_4 to a concentration of 1×10^6 cells per ml. Thirty ml. of the suspension were transferred to a sterile Petri dish bottom and treated

*This paper, illustrated with slides, was presented at a meeting of the Division on March 20, 1959. The work described in this article was supported by grants from the United States Atomic Energy Commission, AT (11-1) 424, Washington, D.C., and the Damon Runyon Memorial Fund, DRG 407, New York, N.Y.

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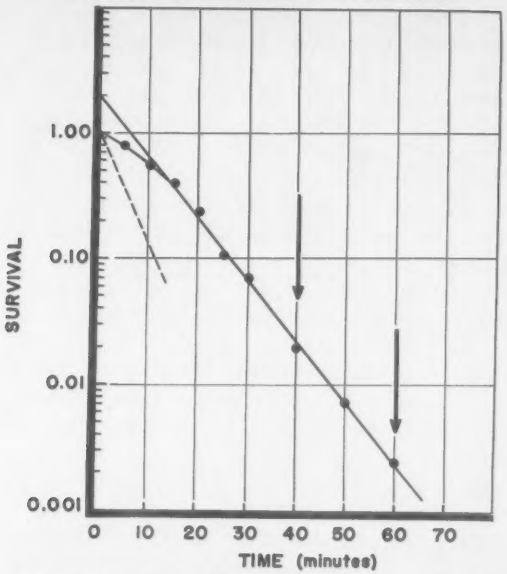

FIGURE 1. A graph showing the decimal fractions of a population of a diploid culture surviving different X-ray doses, plotted against dosage on semilogarithmic paper. The X rays are emitted at a rate of 850 ± 50 r per minute (10-min. dose = 8500 r). The straight-line portion of the diploid curve extrapolates to 2. The dotted line is a typical haploid plot and extrapolates to 1. At 8500 r, 10.4 per cent of the haploid culture survives, while about 70 per cent of the diploid population survives the same dosage. The two arrows indicate the doses to which the cultures were subjected before colonies were removed for further testing.

with X rays measured at the level of the suspension with a Victoreen 2500-r dosimeter. A magnetic stirrer insured a homogeneous cell suspension during irradiation. After irradiation for 40 min. at a rate of 850 (± 50) r (total dose, 34,000 r) of X rays per minute, aliquots were sampled and, after 60 min. radiation (total dose 51,000 r), aliquots were again collected. The aliquots of control and irradiated suspensions were diluted when necessary and plated in at least quadruplicate on the surfaces of 2 per cent glucose-nutrient-agar plates. The plates were incubated at 30° C. for 4 days. The criterion for survival was the ability of cells to form a visible colony after 4 days of incubation.

X-ray survival curves of yeast cells of "young" (1 to 3-day-old) cultures exhibit a "tail" at doses yielding less than .5 per cent survival. The "tail" portion of the curve can be ascribed to a few budding cells in the population that are resistant to both inactivation and mutation (Beam *et al.*, 1954; Pittman, 1956). The proportion of radio-resistant cells is lower in old cultures than in young ones (Wood, 1953) and, in 3- to 8-day diploid and polyploid cultures, the percentage of resistant cells is usually less than 0.01 per cent.

In the present study an "old" diploid culture 11294 was irradiated (the curve showed no "tail" [FIGURE 1]) and the surviving colonies were transferred from the treated plates and examined cytologically and subjected to tests for the determination of the degree of ploidy. Twenty clones were derived from the irradiation experiments: 10 clones at a survival level of 2 per cent (34,000 r) and 10 clones at a survival level of 0.25 per cent (51,000 r). The colonies were transferred to glucose-nutrient-agar slants and incubated 3 days at 30° C. These levels of dosage are in the straight-line portion of the curve well below the shoulder (or threshold dose) and at dosages before which the "tailing-off" phenomenon (when it occurs) is observed. Small colonies are often produced by X-irradiation of polyploids; two of the isolates were of this type. None of the small colonies was respiration-deficient. Adenine-dependent (red) variants are produced with high frequency from heterozygous adenine-dependent diploids (Pittman, 1956). One such variant was included among the 20 colonies.

Loss of centriole by X irradiation. The centrioles cannot be counted in every cell brought under observation, for the centrosomes cannot always be found in all cells, although there are circumstances in which nearly every cell shows a centrosome. After the centrosome is found, it is not always possible to determine precisely the number of centrioles that the centrosome contains, since the clarity of the figures depends on many factors. In a critical study of an old (3-week) unirradiated *diploid* culture (TABLE 1) about 60 per cent of the centrosomes identified contained 2 distinguishable centrioles, and about 20 per cent of the centrosomes contained 4 centrioles, obviously in "telophase." The inference that diploids contain 2 centrioles was justified on the assumption that conditions for observation are not optimal in most cells. About 60 per cent of the centrosomes in a standard untreated 2-day-old unirradiated *haploid* culture contained only a single centriole, and 25 per cent of the cells contained 2 centrioles in the single centrosome. It is inferred that the centrosomes containing 2 centrioles were probably in haploid cells in which the central body was in "telophase." The degree of ploidy of untreated cells can be determined by examining cells cytologically and counting the centrioles; most of the cells show a number of centrioles corresponding to the degree of ploidy, while about one fifth of the central bodies, even in resting cells, will be in telophase. The dotted line in FIGURE 1 indicates that, after 10 min. of irradiation at 850 r (total dose 8500 r), 10.4 per cent of the haploid cells may be expected to survive. The curve drawn through the solid circles is the curve of inactivation of a diploid culture with an extrapolate of 2. After about 10 min. radiation at 850 r (total of 8500 r), about 70 per cent of the diploid cells survive. TABLE 1 shows that 70 per cent of the untreated diploid cells (which contained 2 centrioles per centrosome) survived 8500 r, and 10.2 per cent of the untreated haploid cells (which contained 1 centriole per centrosome)

TABLE 1

NUMBERS OF CELLS CONTAINING SPECIFIED NUMBERS OF CENTRIOLES IN UNTREATED CULTURES TOGETHER WITH THE PERCENTAGES OF SURVIVAL AT 8500 R AND THE KLETT READING FOR A SUSPENSION OF 10^7 CELLS

		No. of centrioles	Percentages of survival at 8500 r	Klett reading per 10^7 cells
		0 1 2 4		
Untreated diploid 11294	Not counting "telophases"	53 58 189		
	Counting "telophases"	16 32 120 35		
	Total	69 90 309	70.0	83.8
		No. of centrioles 0 1 2		
Untreated 2-day-old haploid	Counting "telophases"	24 126 50	10.2	40.4

survived. These cultures obviously satisty the respective radiation criteria for haploidy and diploidy. Another criterion for ploidy developed by Ogur (1955) is the Klett reading per 10^7 cells. Suspensions of both the untreated diploid and the untreated haploid cultures were made and adjusted to 10^7 cells per ml. and the readings were respectively 82.8 for diploid and 40.4 for haploid, indicating that these cultures satisfy this criterion also.

The 20 cultures surviving X-ray treatment were transferred to agar slants and the number of centrioles per centrosome was determined for each culture. All the clones derived from the survivors of X-ray treatment gave the haploid count: about 60 per cent of the centrosomes contained only a single centriole and about 20 per cent contained 2 centrioles, but none had 4 centrioles, indicating that the survivors of diploid cultures treated with X ray have only a single centriole (TABLE 2). These data suggest that radiation inactivates the centrioles independently of each other and that the inactivation of 1 centriole in a diploid produces a viable cell with a single centriole, also that inactivation of the remaining centriole will render the cell incapable of producing a viable colony. After irradiation, many of the cells grow to produce microcolonies but fail to grow further, suggesting that disorganization resulting in eventual lethality may not be an immediate event.

The percentage survival of the different cultures at 8500 r was determined. Two cultures gave haploid values of 10.2 and 10.4 per cent, respectively. Two others gave values much lower than the diploid, but not quite approaching haploid, values, while all the other cultures gave

TABLE 2

NUMBERS OF CELLS CONTAINING SPECIFIED NUMBERS OF CENTRIOLES PER CENTROSOME IN SURVIVING CULTURES OBTAINED FROM ORIGINALLY DIPLOID CULTURE 11294 AFTER IRRADIATION, TOGETHER WITH THE PERCENTAGES OF SURVIVAL AT 8500 R AND THE KLETT READING FOR A SUSPENSION OF 10^7 CELLS

	Treatment 34,000				Treatment 51,000		
Culture number	Nos. of centrioles 0 1 2	Percentages of survival	Klett reading	Culture number	Nos. of centrioles 0 1 2	Percentages of survival	Klett reading
1	15 40 8	70.5	85.3	1	14 37 14	10.2	59.4
2	26 36 10	10.4	69.7	2	8 37 11	48.9	73.2
3	10 35 12	95.2	79.8	3	11 31 10	57.7	80.9
4	11 50 4	80.9	73.7	4	11 35 12	27.4	74.4
5	1 7 0	78.9	88.2	5 (Small)	18 27 10	89.3	84.1
6	8 46 6	75.4	72.8	6	10 43 4	78.8	65.8
7 (Small)	10 37 12	88.0	78.1	7 (Red)	11 31 17	79.0	93.0
8	11 37 11	48.7	82.7	8	5 36 9	23.6	76.9
9 (Small)	14 31 8	57.0	83.9	9	12 28 13	17.7	68.2
10 (Small)	16 32 11	80.0	103.8	10 (Small)	18 48 16	80.7	97.8

approximately the diploid values. The Klett readings per 10^7 cells per ml. were generally in the diploid range. It is clear, therefore, that although each centrosome contained only a single centriole per cell, the other criteria of ploidy (survival at 8500 r and the Klett readings) indicate diploidy. The counts of the centrioles were made shortly after the original treatment and, about one week later, after they had been made, the radiation and the Klett reading criteria were determined. The experiment shows that, although irradiation of a diploid destroys one centriole, it does not automatically convert a cell to the haploid state. This observation is similar to that made by Tobias (1952) who says: "For 6 months experiments were carried out on diploid colonies grown from preirradiated single diploid cells. A number of colonies were found to show defective survival curves. These colonies are demonstrated easily because the initial slope of their radiation-survival curves differs from zero, which is the slope of normal diploid colonies . . . . However, none of these colonies retained their defective properties for more than 5 weeks. Some mechanism appears to be present which allows regeneration of normal diploid cells, which then outgrew in numbers their recessive sisters."

Radiation inactivation curves in Saccharomyces. DeLong and Lindegren (1951) reported that the survival curves from ultraviolet inactivation radiation experiments of the polyploid series of *Saccharomyces* yield extrapolates with integral values approximating their respective degrees of ploidy. The conditions under which their experiment was performed were not precisely specified, and subsequent experiments in this laboratory failed to confirm their finding until it was realized that the cytoplasmic reserves of ribonucleoprotein might interfere with the effects of ultraviolet radiation. When this was realized, cells of the polyploid series were treated with ribonuclease and irradiated with ultraviolet. Integral values of extrapolates were obtained, suggesting that the original experiments by DeLong and Lindegren had been performed with dissimilated cells in which the reserves of ribonucleoprotein were at a low level.

Iguti (1956) has recently shown that haploid cells of *Saccharomyces* and of *Torula* (presumably haploid) gave exponential survival curves with ultraviolet. Commercial RNA at a concentration of 0.1 per cent afforded protection against ultraviolet inactivation of both haploid and diploid cells. These results indicate that cytoplasmic ribonucleoprotein provides a protective screen that absorbs the ultraviolet. This conclusion is consistent with the findings that cells irradiated at high concentrations are more resistant to killing than suspensions of low concentrations. The screening effects of RNA do not interfere in X-ray experiments, and integral values of extrapolates are routinely obtained with cultures containing considerable reserves of RNA.

Summary

The observations reported here indicate that the centrioles are independent, morphologic units that are reduced in numbers by irradiation, and they suggest that the centrioles are the independent, homologous targets, the inactivation of which leads to integral values of the extrapolates of survival curves.

Although these experiments, which relate the effect of irradiation to the number of centrioles among the survivors of irradiated diploid resting cells, are conclusive with respect to resting cells, they do not apply to rapidly dividing cells in which the data indicate many targets in each cell, any one of which may lead to the inactivation of the organism. Thus, in rapidly dividing cells, the centriole is probably not the primary target which it apparently is in the resting cell.

Based on the multihit hypothesis (Atwood and Norman, 1949), the survival curves would indicate that there are 4 targets in a tetraploid, 3 in a triploid, 2 in a diploid, and 1 in a haploid. The 4 integral values are assumed to correspond to 4 morphologic entities within the cell that are independent of each other, the destruction of one in no way affecting the continued survival of the others.

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DIVISION OF ANTHROPOLOGY

CRACKER CULTURE: A SOCIAL ANTHROPOLOGICAL STUDY OF "GEORGIA TOWN"*

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The Southern Region

It is a fallacy and also a misleading stereotype to view the southern states in monolithic terms. Literally, many little Souths make up the southern regions. This multiculture notion of the South was developed by the late Howard Odum and his students. In his classic and period-marking volume *Southern Regions of the United States* Odum stressed the major subcultures of the South and demonstrated that these subregions are not compatible with the political areas labeled the Southern States.

For example, large sections of each of the political areas (states) known as Georgia, Alabama, Mississippi, and South Carolina contain as many as 4 to 6 subcultural configurations. In fact, Woofter,[1] Vance,[2] Gillin,[3] and Odum[4] and others have attempted to distinguish as many as 40 subcultures in the South; Woofter has distinguished 20 in the southeastern states.

For convenience and, at the risk of oversimplification, it is possible, at least taxonomically, to group the large number of small subcultures of the southeastern geographical subregions into 4 major cultural configurations:

(1) *The Lowland Atlantic and Gulf Coastal Plains.* This area includes the so-called Black Belt of the Mississippi Valley in which slavery and the plantation-feudal system had its strongest hold; here vestiges of feudalism are still plainly visible. The caste etiquette, supported by a system of social and sacred logics—folkways, mores, and law—is designed to keep field hands in subordinate positions. This is the area in which there are powerful social and economic forces that buttress the social and psychological climate for rigid segregation, codified by religion, ethics, aesthetics, and law.

(2) *The Piedmont Strip.* Lying immediately above the Black Belt, this area contains most of the South's industry and large cities. This subregion is characterized by greater urbanization, mobility, and social change; also, trade and industrial unionism has penetrated the area. The prevailing agricultural pattern is one of family-worked farms rather than plantations, which are few in number. Moreover, slaves and indentured servants were fewer in number, and local traditions insist upon less rigidity in the separation of humans than in the Mississippi Valley and on the Gulf Coast plains.

*"Georgia Town" is a pseudonym for an actual community located in southeast Georgia in the United States. This paper was presented at a meeting of the Division on March 23, 1959.

(3) *The Appalachian Hill and Mountain Country.* This is the historic domain of the Scotch-Irish, supported by a Presbyterian view of Protestant ethics; the people tend to be conservative in matters of social custom, and liberal in politics. The area has a very weak background of slavery, and questions regarding sociocultural etiquette are markedly different from those of other areas.

(4) *The Piney Woods.* Herein referred to as Cracker Country, this region is an area of the longleaf pine that led the early settlers to refer to it as the Pine Barrens. Its subculture rests upon a "frozen frontier" or a relatively "static technology." In this area, field hands and workers have been dispersed throughout the population, and their ratio to white settlers and planters has always been small.

Cracker Country

It is the purpose of this paper to describe in broad terms the general cultural configuration of this subcultural area that extends from the south central portion of South Carolina through the central part of Georgia, on into north central Florida, and west into south central Alabama, northern Mississippi, and northern Louisiana. The inhabitants living in this area refer to themselves and, indeed, are referred to by others as Crackers. Thus, the term cracker culture is used here to denote the particularities of the culture of these citizens of the United States. Cracker culture, for our purpose, becomes a theoretical construction for describing and analyzing the social structures found in communities located in this exciting subculture of the southern regions.

Cracker culture, therefore, a subtype within the larger regional culture denoted as southern, is distinguishable by three factors: its geographic features (topography, flora, fauna, soil type, and weather); the social logics of its social order (social organization); and the sacred logic (rationale) that supports and gives meaning to the culture.

The outstanding geographical features of the area are its mild winters, hot humid summers, and the red clay upland soils; the latter are the sedimentary soils of the terraces that resulted from the progressive uplift of the Atlantic littoral. They are the sand and sandy-clay loams whose primary drainage system consists of the east-west rivers, such as the Savannah, Satilla, Altamaha, and the Ogeechee, originating above the fall line in the uplands. The rivers serve as channels of migration from the sea-island region of the coast into the fertile areas of the uplands and Piedmont. The relatively infertile soils between these rivers discouraged settlers in search of the wealth that came from cotton production.

Three conflicting views were uncovered for explaining the origin of the term cracker. While it is the contemporary name improperly given to "poor whites" in Georgia, Alabama, Mississippi, and Florida, Burke[5] says: "These people, who live in the manner described, are known by the name

crackers, so called from the circumstance that they formerly pounded all their corn, which is their principal article of diet. It was done by placing the corn on a flat rock, and then beating it with another, but now the hand-mill is used by many, which facilitates the process of cracking the corn, although the meal made by the mill is not much finer. There are but a few watermills in the south part of Georgia, owing to a want of falls."

On the other hand, Buckingham[6] states: " ...we saw many of the country people coming into town .... They were in general as primitive in their dress as the farmers of the remotest parts of England and Wales a century ago.... They are called by the townspeople 'Crackers,' from the frequency with which they crack their large whips, as if they derived a peculiar pleasure from the sound...."

John Maclaclan of the Department of Sociology, University of Florida, Gainesville, Fla., said in an interview that the term, of Seventeenth Century English derivation denotes "bomb," which is used occasionally in the rural South today to characterize a person of explosive temperament. Maclaclan suggests that it may refer to the explosive personality types of the Seventeenth Century frontier.

There are few if any scientific studies that have described the "cracker culture area" as a special region. While there have been both fictional and nonfictional studies of the area, few that have come to my attention attempt to treat it as a special cultural subtype. The best known novelist who has dealt with social life in "Crackerland" is probably Erskine Caldwell. His *Trouble in July*[7] depicts the dynamic processes involved in a lynching. In *Journeyman*[8] he characterizes the itinerant minister and, in *God's Little Acre*,[9] he presents a picture of the familial and sexual adjustments of a "poor white" family. Caroline Miller's Pulitzer Prize-winning novel, *Lamb in His Bosom*,[10] is a historical sketch of the process by which her heroine is assimilated into the society of the piney wood folk. Cecile Marie Matschat, in *Suwanee River, A Strange Green Land*,[11] has defined in some detail the way of life of the people of the Okeefenokee Swamp; Vereen Bell, in *Swamp Water*,[12] dealt with similar themes. Zora Neale Hurston, in *Seraph on the Suwanee*,[13] portrayed the ideal-type behavior of the subservient Negro. In a little known novel, *Suwanee Valley*,[14] Borchardt and Sears reveal the life of the "cracker folk" in and around the town of Old Troy, Lafayette County, Fla., during the late 1890s and early 1900s. It is perhaps the only work mentioned here that gives insight into the archaic political processes of the controversial county unit system of government presently in operation in the state of Georgia. Marjorie Kinnan Rawlings, in *The Yearling*[15] and *South Moon Under*,[16] presents pictures of family life in the Big Scrub country of Florida. Her *Cross Creek*[17] is an admirable picture of life and character types of the Cross Creek area of Alachua County, Fla.

Georgia Town

Cracker culture, as employed in this paper, is not only a heuristic device that allows us to think in broad concepts about contemporary social life, but it is one that, I believe, has extensions into reality. While none of the communities in the area is the exact replica of the configuration described, each is a close approximation. Robert Redfield,[18] Howard Becker,[19] and Max Weber,[20] among others, have described the value of the "ideal types" as research tools for social scientists. Therefore, Cracker culture, for operational purposes, is simply a constructive typology for cultural analysis.

Georgia Town was selected for intensive study after considerable reconnoitering throughout the area. The community is at the very center of cracker culture; it reflects the tradition; also, it serves as an excellent example of a community whose culture and social organization most nearly fit our ideal conception of "Crackerland."

Economically, Georgia Town is a market center, a key "trading town;" politically, it is a county seat of an important rural unit of the state. The community has a population of approximately six thousand, of which 30 per cent is Negro. As a market center, it serves a large farming area; in recent years, the introduction of automobiles and trucks, and with them all-weather roads, has extended its trading area to surrounding counties.

Cracker County has both a recent history and a long tradition. The area was first settled in 1740. By 1790 it was incorporated as a county by the Georgia State Legislature. Until 1830 its county seat was located at a point where the traveling court convened. However, in 1830, Georgia Town was designated as the county seat and, by 1880, the town was composed of a courthouse, a hotel, a general store, a jail, and six residences.

Since 1880, Georgia Town has grown slowly, unevenly, but steadily. As indicated earlier, it is located at the very vital center of an expanding agricultural area. The stimulus of governmental activities in agriculture, the mechanization of agriculture, and the corresponding increase in the size of farm units have resulted in a decrease of the need for agricultural workers, thus producing a greater number of nonfarm ruralites in Cracker County. Although the county was originally settled by single family-farm units that helped to produce cultural isolation, the single-family farm is becoming increasingly obsolete as a self-sufficient, economic unit.

The historical processes of most cultural areas in the United States usually reveal an increasing population density, an expansion in land use, a rapid transition from a subsistence to a market economy and, finally, the proliferation of market and trade centers as well as nonagricultural industries. Such technological change is usually accompanied by increasing complexities in social structures with a corresponding secularization of the "social logics" to support appropriate behavior patterns. The paucity of natural resources, the lure of better public lands in the West.

and the original settlers' lack of technological skills and resources in this area resulted in the avoidance of the area by the main streams of migration.

Patterns of Culture

However, with a tradition of geographical isolation, plus the endogamous relationships of isolated groups, an extended kinship group has developed in which the importance of family sentiments as a basis for the social organization still persists. Moreover, the religious beliefs of the town are centered in a fundamental Protestantism based on sacred writings assumed to embody all wisdom and knowledge necessary for the proper ordering of man's behavior. The unit of worship is the congregation, and the church itself simulates into a large household group. The members are joined by spiritual ties of "brotherhood"; it is common for church members to refer to each other as "brother" and "sister," almost invariably in the church itself and quite often in daily life.

In respect to its biracial social structures, Georgia Town might be contrasted with a small town in the "Plantation South." In the latter, white planters conceive of their superiority as the result of biological and, more recently, cultural superiority, while the Cracker sees Negroes and sometimes what they call "poor white trash" as inferior because of filial disobedience. For example, some Georgia Town "primitive Baptist" churches regard Negroes as inferior because presumably they are descendants of Noah's grandson Ham who, because of lack of respect for his grandfather, was punished by God's injunction to him and his descendants to be the servants—the drawers of water and the hewers of wood—of his respectful older brother's descendants.

The family is the basic productive unit in Georgia Town; each individual family member is assigned specific productive functions; the cash crop (cotton and tobacco) is sold at one time, and money is seasonal. There is, moreover, a marked sexual division of labor, supported by religious sanctions. Economic tasks are apportioned according to age levels, maturation being accompanied by increased participation by the individual in economic activity. The distribution of goods and services is based almost entirely on family relationships.

In contrast to the pattern of ownership and management in the plantation economy, the culture of the Georgia Town family farm accentuates greater isolation of Georgia Town families. Traditionally, individuals who accumulated large tracts of land tended to acquire widely separated farm units rather than contiguous areas of land operated by a community of tenants. The large landholders did not acquire the traditional status associated with plantation ownership, nor did they have the financial and political power and the social prestige of the planter class in other subregions. Perhaps this partially accounts for the crucial features of the culture and explains the persistence of its folklike character.

To a very great extent, the culture is child-centered: children are assigned specific economic roles in the family and are treated with intensive delicacy and affection. They are accepted as the natural consequence of the marital relationship. The grandparents are the chief sources of affection; they are permissive figures and the source of affectionate discipline. The parents are stern, albeit affectionately so; they are nonpermissive, constraining, yet consistent. The rigidity of Cracker parents is but a reflection of the social sanctions that mark this culture. Moreover, the blind obeisance of the child to this culture is taught systematically and with painstaking care, the results of which are manifested in the child's apparent inability to act with initiative or to engage in introspective and reflective thinking about his parents' behavior.

In this connection, the individual seems to be at a loss in moments of crisis, that is, if the culture does not provide him with patterns of responses, or if he cannot integrate new patterns that are introduced into the culture. For example, the parental generation still looks to the child's grandparental generation for direction, emotional support, and guidance. Accordingly, the source of authority in the child's world is usually both parental and grandparental. As a child grows up, authority is not repeated by a single personality or even by his immediate family, but by many adults of the community. This may suggest, at least partially, the psychogenesis and sociogenesis of the crowd-mindedness of Crackers, as well as the mechanisms through which both Negro and white children learn to accept all adults as authority figures in this cultural configuration.

While the exact symbolic significance of the kinship relationship as denoted by the term cousin is not readily apparent, it is applied indiscriminately both to those individuals who stand in close kinship relations, both maternally and paternally, and those persons who are descended from a common known ancestor, as well as those who bear the same family name. It is not unusual for the thoughtfully aggressive and socially mobile person to admit to as many as two thousand relatives within the bounds of one county. This appears to have important implications for economic, cultural, and political behavior. In fact, extended kinship ties are very useful to politicians for uniting a series of presumably related persons into effective political action groups.

Usually an extended family system consists of a core of "close" kin and an amorphous group of "distant" kin. While blood relationship is naturally the rationale for these kinship groups, a cultural definition of kinship expressed in terms of close and distant depends upon the kinds and degrees of participation in the family social structure. Thus, it is not infrequent to find individuals in Crackerland using a technique of claiming kin. Thus, the over-all social structure rests on the establishment of interrelationships of members of one close kin with others by the development of friendship, marital loyalties, and implementation of distant kin ties. Accordingly, the social organization of Georgia Town for group ac-

tion tends to be stable, and can be modified only as the interrelationships between various groups of close kin become knitted together into new dimensional structures for social action.

Religion penetrates every facet of institutional life in Georgia Town; it serves to facilitate the orientation of the individual to his culture. A religious unit that consists of a congregation, its members and believers, has two holy possessions—the church edifice and the Bible. The Bible serves to provide moral sanction for the norms of the culture. Such life conditions as sex roles, family stability, filial respect and obedience, child discipline, and white supremacy are supported by divine law as interpreted in the church by the minister and his congregation.

The principal denominations in this area are Methodist, Missionary Baptist, and Primitive Baptist. There are few other denominations with any sizable membership. While the Primitive Baptist is the smallest of the three denominations, Cracker culture is one of its strongholds. The main tenets of this creed are that the social order represents divine intervention. The world is predestined; this creed may account for the Cracker's lack of guilt feelings or perceptions of personal responsibility whenever he becomes explosive and abusive to others.

Education in and of itself is no qualification for the ministry. God is seen as a father who decrees rules adhered to out of filial respect. The minister is frequently a deviant; he is often "possessed" when he interprets the Gospel in his sermon; frequently he speaks in unknown tongues and spasmodically jerks as though he were in a hypnotic state or having an epileptic seizure. Conversion in the traditional sense of the perception of guilt, the public confession, and the public welcome of handshaking is absent among the Primitive Baptists; it occurs only when the individual gives some outward indication of divine grace—a feeling of salvation, a vision, or mark. Conversion and church membership occur during early adolescence and mark the assumption of adult status by the individual.

In Cracker culture, "God created man to enjoy dominance over the beast of the fields." The church edifice is the house of the congregation presided over by the Father, and, by semantic inference, represents heaven. Thus, "In my Father's house are many mansions...." The sacred logics[21] of the church are identical to those of the family system: the church building represents the family home; the Bible represents the moral prohibitions of the father. For example, sex roles are restricted in that all congregants are brothers and sisters; sex behavior is regulated by two polar personality typologies—Adam and Eve in contrast to Joseph and Mary Magdalene. Within these polar extremes, all sexual behavior becomes lust, especially if carried on by middle-aged parents. Approved sexual behavior is confined to procreation, but even then "all humans are born in original sin."

Cracker Culture, Personality, and Negro-White Relations

Slavery was well established as a southern institution during the development of Georgia Town. However, Negro slaves were concentrated in the expanding plantation regions of the more fertile soils of the interior. Accordingly, race relations can be understood in Crackerland only when it is remembered that Negroes were dispersed among the white population, which at all times outnumbered them. Negroes and whites lived in relatively intimate social contact; work parties and other symbiotic groups often consisted of several white persons and a few Negroes. The close interpersonal relations between the two racial groups resulted in the acquisition of common sets of cultural norms. Because of the cultural context in which Negroes have come to accept these values, those who remained within the area and maintained close contacts with whites acquired accommodative orientations and techniques appropriate to the culture. Those unable or unwilling to establish intimate personal relationships with white employers or "friends" found it difficult to function effectively, either within the context of the cultural configuration or even within the Negro group.

Custom requires a Negro to have a white sponsor who will relate him to the white community. Both concrete services such as cooking, housekeeping, and baby sitting, and evidences of respect, obedience and deference are expected as expressions of gratitude for this sponsorship. Correspondingly, Negroes who maintain economic relationships with whites expect sponsorship as part of the "contract." Even Negroes in the professions and business must make every effort to establish themselves with a white protector. However, the cultural emphasis is upon the informal and personal content of the Negro-white relationship.

There can be found implicit in race relations in Georgia Town much interpersonal violence, taking the form of both inter- and intraracial conflicts. However, the amount of violence within the white group is seldom as great as that among Negroes. Moreover, there is a qualitative difference of kinds of conflicts between the two groups. The difference might be explained by the fact that Negroes at any time may be intimidated and humiliated by whites, but those who have been residents of the community for a long period become accommodated to these forms of interaction; they treat them as expectational behavior. They resent only the unusual and the unexpected forms of subordination. Whites not infrequently confront their Negro clients with the fact that they have failed to live up to the sanctions and expectations of the culture; this serves to keep the Negro community divided and generates individual anxieties. When these frustrations reach the point that they must be expressed in overt aggression, Negroes have learned that it is safer to express them within the Negro group than across racial lines.[22] It is within this configuration that Cracker communities make use of the psychodynamics underlying frustration and aggression by providing individuals with conventionally acceptable roles that permit them to release unconscious impulses within the frustrated group.

Mob violence is more often a social occasion, a ceremonial event, in which sentiments about, and attitudes toward, the subordinate outgroup are expressed and taught. In plantation society, however, group violence is essentially a means of social control of its slave and peonage labor by a small white minority. Thus, in the Deep South mob violence such as a lynching takes place without much public clamor and makes little impression upon the plantation community.[23] On the contrary, in Cracker culture there is usually a shared community feeling, a consensus as it were, involved in the "lynch situation." So long as there is a scapegoat available, the actual victim is of little importance. Violence in the area may be viewed, not only as an enforcement of caste regulations, but as an expression and reinforcement of community values.

Social Change and Emerging Structures

The cultural configuration of Crackerland is far from a statis phenomenon; the area is undergoing rapid, almost revolutionary changes in its traditional cultural patterns. The Crackers are developing traits that are manifested in a secularized culture, and the folklike, static rigidity of the culture is breaking up and disintegrating under the impact of technology, urbanization, migration, and mass communication.

Perhaps the most poignant changes in the culture relate to the rapid upsurge of a planter and business class within the region.[24] Federal and state agricultural programs have introduced new cash crops into the area, broadening the scope of productivity and marketing efficiency of the society. The subsistence nature of the economy has given way to a money economy, thereby modifying the paternalistic-landlord-tenant-sponsorship-client relationships in interpersonal contacts.

Although religion still remains a powerful force in the culture, the rapid rise of marketing activities heralds the appearance of new ethical and ideological bases for religious behavior. The proliferation of new Protestant denominations, the Catholic parish, and the Jewish synagogue is but another manifestation of the emergence of a new associational type society in Crackerland. Moreover, the development of associational groupings is beginning to dispel the complete dependence of Negro clients upon their white sponsors. There is emerging a new racial ideology, accompanied by changing images and sensitivities of the people, both Negro and white.

In one respect, the new forms of race relations in this biracial, caste-like society tend to widen the social distances between the two groups; however, this appears to be only of a transitory nature, while the two groups work out new accommodational relationships.[25] At least, such voluntary associations as the Ku Klux Klan and White Citizens Councils, Parent-Teacher Associations, as well as the C.I.O., the Civil Liberties Leagues, the National Urban League, the National Association for the

Advancement of Colored Peoples, the Anti-Defamation League, B'nai B'rith, and other pressure groups must now operate overtly, out in the open, and offer better than superficial solutions to the requirements and new expectational demands of a society that is incipiently beginning to throw off its traditional cultural patterns. Finally, Crackerland is beginning to develop incipient and naive political forms, processes, and programs that tend to interconnect it with surrounding areas; this growing interdependence means that the political appeals of Cracker politicians can no longer be confined solely to the symbolisms and semantics of Cracker culture.

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DIVISION OF OCEANOGRAPHY AND METEOROLOGY

CLIMATE AND CHOLERA*

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Introduction

In the year 1832 The New York Academy of Sciences was fifteen years old. New York City already had 220,000 inhabitants and was fast becoming the business center of the United States. The young nation was growing; the old Dutch trading post was expanding and changing with it; confidence and optimism characterized the period.

However, in the year 1832 New York was a frightened city. The business district and better-class residential streets were practically deserted. Almost one-third of the population had fled.[1] In the poorer sections despair and terror prevailed. The city of New York was beset by epidemic cholera.

It is difficult for us to go back mentally more than 126 years and imagine ourselves living without modern medical and sanitary facilities, when water was supplied by common pumps and waste was disposed of inadequately; when physicians entertained fanciful theories as to the cause of cholera and other infectious diseases and had no dependable remedies for them; when the cry of cholera evoked the same grisly emotions as did the Black Death in late medieval Europe. Our difficulty in reconstructing the situation that confronted New Yorkers four generations ago may be complicated by another circumstance.

We think of the atmospheric sciences--meteorology and climatology--primarily in terms of physics and mathematics, and we may wonder what associations, if any, exist between them and cholera or, for that matter, medicine.

Climate and Medicine

Before proceeding to the principal topic of this paper, a brief review of medical climatology (or human bioclimatology, as it has come to be called) therefore seems appropriate, restricting the historical account mainly to the United States.

The relationship between medicine and climatology began under the most favorable auspices, for Hippocrates is credited with the first discussion of the influence of weather and climate on living things, in his treatise on *Airs, Waters and Places*, written about 400 B.C.[2,3] The "Father of Medicine" also set the tone for treatment of the subject during the following 2200 years. Throughout this entire period almost the only factors taken into account in order to explain disease were such environmental (includ-

*This paper was presented at a meeting of the Division on March 24, 1959.

ing atmospheric) factors as the purity of the air, the nearness to water surfaces, and the local topography.[4] In 1676 Thomas Sydenham, the famous English physician, systematized the old ideas into a theory that divided all acute diseases into two classes.[3,5] He attributed "stationary fevers" or "epidemic distempers" to mysterious "effluvia" emitted into the air by "hidden and inexplicable changes" in the depths of the earth. Of the other class, "intercurrent or sporadic fevers," he wrote that they "originate most especially in this or that manifest atmospheric temperament;" for example, "pleurisies, quinsies and other like ailments, generally prevail when long and severe cold is rapidly succeeded by sudden heat."[5]

In the Nineteenth Century medical men continued their traditional interest in climatology; for example, in 1866 the American Medical Association appointed a committee for the "investigation of the effect of climate and weather on health."[6] Meanwhile, advances in medicine in other directions were climaxed by the discoveries of Louis Pasteur beginning in 1873, which "diverted the attention of interested scientists from the important study of man in his environment to that of bacteriology and allied sciences." Only recently has interest reawakened in the relationship between climatic factors and human health and disease.

Medical climatology is thus both a very old and a very young science. The same may be said of climatology itself, for its origin is likewise traceable to ancient Greece, Eratosthenes (*fl. ca.* 200 B.C.) probably being the first to organize the major climatic controls into the familiar scheme of the torrid, temperate, and frigid zones.[7] Little was added to this rudimentary scheme until the beginning of systematic weather observations in the latter half of the Eighteenth Century.

More will be said later about the present status of medical climatology. To stress its primitive state when cholera struck New York in 1832, on neither the medical nor the climatological side was there adequate scientific information. A connection between disease and atmospheric conditions had long been supposed, but beyond this "the doctors disagreed." In addition, and perhaps as a consequence of the medical preoccupation with atmospheric influences, scant attention was paid to pure water supplies and sanitary sewage disposal. As New York and countless other communities discovered, the results were catastrophic.

Cholera in New York

The disease has been endemic in certain parts of India since time immemorial, but it is controverted whether it afflicted other countries (except possibly China) until the early Nineteenth Century.[8-10] Starting from its source regions in 1819, under the name of the Asiatic or Indian cholera, the disease spread westward in two waves across Asia and Europe to reach the British Isles about 1830 to 1832.[8] It crossed the Atlantic to New York with emigrants from Europe.

The open outbreak of cholera occured there about July 1, 1832. The peak came 3 weeks later, when 104 victims died on a single day (July 21) and 295 of 778 cases expired during the surrounding 3-day period. By the end of July, the daily death toll stood at 48 and total deaths numbered 2085. At the close of August, the death rate had declined to 30 per day; a month later it was down to 7 and the epidemic ended about mid-October.[1] In a little more than 3 months it had taken 3513 lives. The over-all death rate for 1832, almost 50 per 1000 population, was unprecedented in the city of New York since the beginning of mortality statistics in 1805 and has since been approached in only 2 subsequent "cholera years".[11] In Brooklyn, N.Y., an independent city until 1898, cholera claimed 274 of 17,000 inhabitants.[12]

Most of the Eastern states, including the inhabited portions of the Mississippi Valley, were affected by epidemic cholera during the years 1832 to 1835. It struck New York again in 1834, taking 971 lives.[11]

In 1832, to quote J.S. Chambers, historian of the disease in the United States, the best majority of physicians there still believed in the theory developed by Sydenham 150 years before: "They thought that the pestilence was caused by a poison in the air – effluvia, miasms, malaria – which rose from the 'bowels of the earth,' from decaying animal or vegetable matter or from the bodies of the unclean or the sick."[1]

The second epidemic in the United States came in 1849 and spread even farther over the country during the next four years than had the first outbreak. The Gold Rush increased travel and helped to facilitate transmission of the disease. Although other places suffered even more severely, the city of New York nevertheless lost 5071 of one half-million inhabitants,[11] and Brooklyn 650 of 90,000.[12] Locally, the epidemic spread slowly during May 1849, intensified during the next two months, reached its climax about the first of August, and ended in September.[1]

By 1853 the second epidemic in the country seemed over; then the third outbreak arrived. In New York and Brooklyn, the cholera began about the end of May 1854. It developed rather slowly during June and early July; its peak extended from July 23 through August 19, when the daily death rate averaged nearly 40. It declined gradually thereafter and did not end until early December.[1] It took 1137 lives in New York[11] and 678 in Brooklyn.[12] For 1849 the New York over-all death rate was close to 49 per 1000; for 1854, it was about 47. These are the only 2 years that rival the terrible losses of 1832.

In England, 1854 was also a cholera year. A London physician, John Snow, proved that the center of infection in his neighborhood was contaminated water from a street pump.[3,13] The Royal College of Physicians investigated, deliberated, "patted him on the back and told him that he was a good boy, but declared it most likely that the cholera poison was carried from place to place on the wings of the wind."[1]

The disease returned to the United States in 1866, when at least 1137 died in New York [11] and 800 to 900 in Brooklyn.[1] This locality and the East in general were hardly affected in 1872-1873, when a major outbreak swept the Southern and Mississippi Valley states. Even at this date some doctors adhered to the miasma theory.

Such views, however, had been subjected to serious criticism. Chambers states that in 1866, while "there was still much talk of miasms, malaria and the air" among physicians in the United States, "there was also much discussion about transportation, contact and contamination; even the word germ was occasionally used." Somewhat earlier, Lorin Blodget made some shrewd observations in the chapter on "General Sanitary Relations of the United States Climate" in his *Climatology of the United States* (1857):

"Cholera is more obscure in its climatological relations than any other disease of similar violence, and what its associations are it is very difficult to say.... It might be supposed to follow certain tracts of country when communication favored, and at a certain temperature, but as yet no decisive restrictions can be assigned....

"It may be safely said, that if cholera was originally a disease of climatological character and limits, it has now become something more, and, as known to us under the name of Asiatic cholera, has much of the character of a poisonous infection. Climate is, in the temperate latitudes, most signally the incident and modifying agent, and high temperature with great humidity the particularly favorable condition."[14]

In reaching this conclusion, Blodget was influenced no doubt by a striking incident that he relates in the same chapter. A Buffalo, N.Y. physician noted that, during the 1854 epidemic there, most of the deaths occurred on a day when the dew point reached 77.2° F.; that mortality was great while high temperature and high humidity prevailed; and that when the dew point fell to 63° F., *"from that hour the epidemic ceased."*[14]

In this connection, it is interesting to examine some climatological and mortality records for the 1854 epidemic in New York and Brooklyn which, it will be recalled, extended from late May to early December. FIGURE 1 is based on observations taken at Fourth Street, near Lafayette Place, in New York, by Ruston Maury.[15] For each week ending on the date indicated, the extreme maximum and minimum readings and the weekly mean temperature are given, together with the number of cholera deaths. It will be seen that the temperature curves and the weekly death totals are in generally good agreement. Particularly striking are the 4 weeks from July 23 through August 19, when 1488 died and the temperature never fell below 63° F. Maury's mean temperatures for the July-August weeks are about 2° higher than the monthly means recorded at Fort Columbus, but agree closely with the Fort Hamilton means for these months.[16] Maury's extreme temperatures are almost the same as theirs for the same months, and receive some support from Spader's contemporary but incomplete temperature

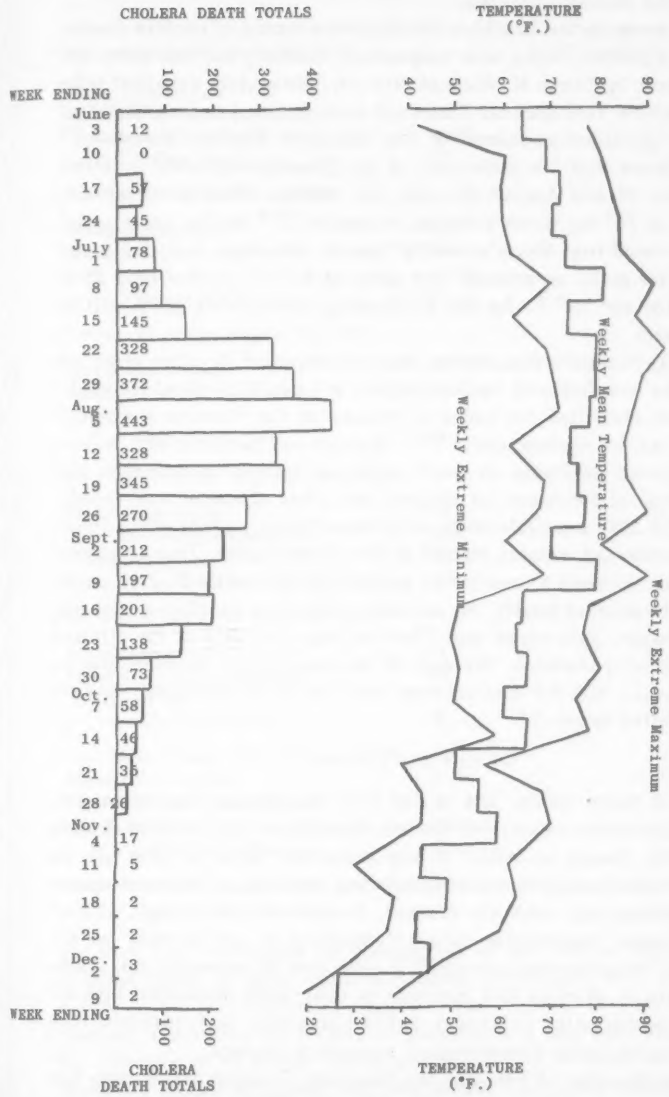

FIGURE 1. Weekly cholera deaths and weekly mean and extreme temperatures, May to December 1854, New York, N.Y.

series at New Brunswick, N.J.[17] In FIGURE 1, it will be noticed that the fall in the weekly temperatures after early September is paralleled by a decrease in the cholera fatalities.

TABLE 1 presents the Brooklyn Health Office record of cholera deaths for the same epidemic, along with temperature, rainfall, and dew point observations made by James M. Minor of that city. These data were first published in the New York Medical Times and were reprinted in the official report of the physician-in-charge of the Brooklyn Cholera Hospital.[12] TABLE 1 shows that the major part of the Brooklyn epidemic occurred between June 25 and August 26, with 531 deaths. Minor gives a mean temperature of 76° for these 9 weeks; a mean of 77° for the same period can be calculated from Maury's weekly figures. Attention is called to the fact that Minor gives an average dew point of 62° F. for the June 25 to July 22 period and 59° F. for the 5 following weeks. This point will be discussed later.

After 1873, Pasteur's discoveries were accompanied by other great advances in the new fields of bacteriological and parasitological research. In 1883 Koch identified the cause of cholera as the "comma bacillus," later known as the cholera *vibrio*.[18,19] Doctors and public health authorities now placed emphasis on good sanitation instead of miasmas, and effective medical treatment for cholera and other diseases was rapidly developed. In 1892 a terrible cholera epidemic began in Asia and Europe. Eight badly infected vessels arrived in New York Harbor. Thanks to proper care, only 44 deaths occurred in quarantine and, while 9 of 10 cases in the city terminated fatally, no secondary infection developed. According to Chambers, this event was "for the rank and file of the [United States] medical profession, the end of the controversy between miasms and microbes.... And the final victory was one of experimental science over speculative theory."[1]

Climate and Cholera

In view of these words, and of the fact that (except for occasional lazaret or quarantine cases) the Western Hemisphere had its last cholera case in 1911, Europe in 1923,[9] it may seem that there is, after all, no significant relationship between climate and cholera, or between atmospheric conditions and epidemic disease. Is medical climatology, it may be asked, mere "speculative theory," deprived of any validity by the triumphs of "experimental science?" A good way of answering this question consists in showing that research is still being conducted into atmospheric relationships to disease and in illustrating such research by a modern approach to the climatological aspects of cholera.

Under the direction of J.M. May the American Geographical Society has undertaken a study of "the geography of disease."[4] Each of the transmissible diseases is regarded as the clinical expression of a complex of pathogens. Cholera exemplifies a two-pathogen complex, which in its

TABLE 1

BROOKLYN HEALTH OFFICE RECORD OF CHOLERA DEATHS, 1854 AND WEATHER RECORDS OF J.M. MINOR, BROOKLYN, N.Y.

Four weeks ending June 24, 1854		*Average for the same weekly periods, 1846 to 1853*
Mean temperature (°F.)	70	69.5
Mean dew point depression (°F.)	14	
Total rainfall (inches)	2.40	3.50
Cholera deaths	33	
Four weeks ending july 22, 1854		
Mean temperature	76	74.5
Mean dew point depression	14	
July rainfall	1.71	
Cholera deaths	231	
Five weeks ending August 26, 1854		
Mean temperature	76	73.8
Mean dew point depression	17	
Total rainfall	1.25	4.10
Cholera deaths	300	
Four weeks ending September 23, 1854		
Mean temperature	71	67.5
Mean dew point depression	11.6	
Total rainfall	2.63	5.50*
Cholera deaths	90	
Four weeks ending October 21, 1854		
Mean temperature	61	59
Mean dew point depression	13	13.5*
Total rainfall	1.05	2.10
Cholera deaths	19	
Four weeks ending November 18, 1854		
Mean temperature	49.5	49
Mean dew point depression	9	8.5
Total rainfall	4.50	3.50
Cholera deaths	5	

*Value for corresponding period in 1853 only (Brooklyn's 1854 population was 200,000).

simplest form represents the conjunction of man with an infective agent. In some cases, of which cholera is again an example, it is not sufficient merely to bring the infective agent and man together in order to create the disease. Environmental factors or geogens must interpose their effects if the infection is to occur. These geogens belong to three broad classes: physical, human or social, and biological. The study under discussion seeks to identify these environmental factors in each of various diseases and determine their effects.

In the case of cholera, the social factors of population density, housing, and sanitation are clearly of prime importance. Positive correlations are given for each of these with both parts of the two-pathogen complex. Among the physical geogens studied are the following climatological elements: sunshine and cloudiness, rainfall and humidity, barometric pressure, temperature, wind direction and speed, and solar radiation. Negative correlations are shown for the first two factors (sunshine and cloudiness). Correlations are listed as doubtful for the last pair (wind velocity and solar radiation) and for atmospheric pressure. In the case of temperature, the correlation is given as positive for the infecting agent and questionable for the human half of the pathogenic complex. It is given as positive for both as regards rainfall and humidity.

Reconsideration of the climatic factors listed by May will bring out the complexity of the problem. First of all, they are experienced as a totality, they are intercorrelated to different degrees, and they occur in varying combinations. It is difficult to isolate the effect of, for example, heavy rainfall from that of high humidity. In the second place, it follows that general assertions and purely statistical statements can be made about the relationship between the climatic elements and cholera, but precise or quantitative evaluations of the relationship are much more difficult to establish.

With cholera, there can be no doubt of the importance of high temperatures, but at what threshold temperature the pathogen passes from the quiescent to the virulent state is unknown. High humidity also appears to be a major factor in promoting the activity of the disease, but its role is not yet defined. The statement that "cholera epidemics occur only during the hot season when there is a vapor pressure of 0.4 in. (14 mb) or more" is open to question. This element is a measure of the absolute humidity, or density of water vapor in the atmosphere. The dew point, or temperature to which air with a given water vapor content must be cooled, at constant pressure, in order to become saturated, is also a measure of the absolute humidity. For each value of the partial pressure of water vapor there is a corresponding dew point; in the present case, 0.4 in. or 14 mb corresponds to a dew point of 52.7° F.

It will be remembered that the Buffalo physician observed that the 1854 epidemic there ceased abruptly when the dew point fell to 63° F. – well above the supposed threshold value. On the other hand, it was noted that Minor's data for the 1854 Brooklyn epidemic (see TABLE 1) gave an average dew point of 62° F. for the first serious period (June 23 to July 22); 59° F. for the next 5 weeks (July 23 to August 26), during which deaths continued high; and showed October and November average dew points, when the epidemic was fading, of 48° and 40.5° F., respectively. However, between August 27 and September 23, when the daily death rate declined to 3, after being close to 9 during the preceding 5 weeks, the average dew point was also 59° F.

It can be objected that these contradictory results are meaningless, since Minor's measurements may have been inaccurate and since, in any case, their "representiveness" or applicability to the places in which the cholera fatalities occurred is debatable. The latter objection holds against the 0.4 in. vapor pressure value. It was originated by Sir Leonard Rogers, a leading British medical authority on cholera in India.

The tabular data given by Rogers relate average monthly cholera deaths per 100,000 with average monthly absolute humidities, both for the various provinces of India. These are large areas; it is not stated what meteorological stations were the sources of the vapor pressure figures, how many were used, where they were located, or whether they changed or were moved during the 45-year period. Under these conditions, the 0.4-in. threshold value seems unwarranted as a physical law, although helpful as an empirical rule. Rogers himself admitted that he could not fully answer the question, "How does low absolute humidity check cholera prevalence?" [20] and did not even conjecture why the disease should break out with absolute humidity above the 0.4-in. vapor pressure level.

Russel and Sundarajan published a statistical study of the epidemiology of Indian cholera in relation to climatological elements. They concluded: "This investigation has shown that it is no longer theory to suppose that climatic factors have a definite relationship with the incidence of cholera in India. The association of high relative humidity with high temperature, accompanied by intermittent rains, forms the most favorable atmosphere for development of the disease." [21]

This statement, with no setting of quantitative limits for the elements involved, recommends itself to the writer as a fair summary of present-day knowledge of the subject.

Conclusion

It was stated at the beginning of this paper that there has been a recent reawakening of interest in medical climatology (or human bioclimatology). This development should be welcomed by representatives of both of the sciences involved. In "Trends in Climatology," an article that appeared in the October 3, 1958 issue of *Science*, H. E. Landsberg ventured this prophecy: "The greatest advances of climatology are destined to lie in the border field of biology, provided an adequate cooperative research program is started. The interactions between the physical changes in the atmosphere and living organisms are too great a challenge to scientific curiosity to remain in a relatively unexplored state. [7] It is hoped, at least it was intended, that this paper may be regarded as an essay in the desired direction, if only because understanding the past helps in undertaking the future.

Acknowledgment

Thanks are due to the library staffs of the New York Academy of Medicine and Rutgers University, New Brunswick, N.J., for courtesy in supplying several of the references cited; to A.S. Kussman of the Weather Bureau Office, New York, N.Y., for critical review; and to David Ludlum, editor of *Weatherwise*, for permission to use material originally submitted for publication in that periodical.

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CONFERENCE HELD

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

INACTIVATION OF VIRUSES

Conference Co-Chairmen:
Ernest C. Pollard
Department of Biophysics, Yale University, New Haven, Conn.

A. R. Taylor
Research Department, Parke, Davis & Co., Detroit, Mich.

PROGRAM

THURSDAY, MARCH 19, 1959

BIOLOGICAL INACTIVATION OF VIRUSES

Session Chairman: W. Henle
Departments of Public Health and Preventive Medicine,
School of Medicine, University of Pennsylvania, Philadelphia, Pa.

9:00 A.M. –
Greetings from the Academy – Robert L. Kroc, Chairman, Section of Biological and Medical Sciences, The New York Academy of Sciences, New York, N. Y.; Warner-Lambert Research Institute, Morris Plains, N. J.

Introductory Remarks – Hilary Koprowski, President, The New York Academy of Sciences, New York, N. Y.; The Wistar Institute, Philadelphia, Pa.

"Neutralization of Viral Infectivity. Characterization of the Virus-antibody Complex: Association, Dissociation and Host-cell Interaction" – Benjamin Mandel, The Public Health Research Institute of the City of New York, New York, N. Y.

"Properdin and the Inactivation of Viruses" – Harold S. Ginsberg and Ralph J. Wedgwood, Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University, Cleveland, Ohio.

Discussant: Chester M. Southam, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

"Studies of the Nonspecific Inactivation of Newcastle Disease Virus by Mammalian Sera" – David T. Karzon and Robert N. Bussell, Department of Pediatrics, School of Medicine, University of Buffalo, Buffalo, N. Y.

"Interference of Newcastle Disease Virus with Neuropathogenicity of Oncolytic Viruses in Mice" – Robert W. Speir and Chester M. Southam, Sloan-Kettering Institute for Cancer Research, New York, N.Y.

CHEMICAL INACTIVATION OF VIRUSES

Session Chairman: A. R. Taylor
Research Department, Parke, Davis & Co., Detroit, Mich.

2:00 P.M. –

"Interaction of Highly Purified Polio Virus with Formaldehyde" – Fred. L. Schaffer, Virus Laboratory, University of California, Berkeley, Calif.

"Investigations on the Use of Beta-Propiolactone in Virus Inactivation" – Gerald A. LoGrippo, Division of Microbiology, Henry Ford Hospital, Detroit, Mich.

"Chemical Inactivation of Foot-and-Mouth Disease Virus" – O. N. Fellowes, Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Greenport, Long Island, N. Y.

"Considerations on the Use of Formalin in Virus Inactivation" – Jonas E. Salk and John B. Gori, Virus Research Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.

"Theoretical Considerations in the Inactivation of Viruses by Chemical Means" – Sven Gard, Karolinska Institutet, Stockholm, Sweden.

FRIDAY, MARCH 20, 1959

PHYSICAL INACTIVATION OF VIRUSES

Session Chairman: Max A. Lauffer
Department of Biophysics, University of Pittsburgh, Pittsburgh, Pa.

9:00 A.M. –

"Theory of Physical Means of Inactivation of Viruses" – Ernest C. Pollard, Department of Biophysics, Yale University, New Haven, Conn.

"Effects of Nonionizing Radiations on Plant Viruses" – A. Kleczowski, Rothamsted Experimental Station, Harpenden, England.

"Effects of Nonionizing Radiations on Animal Viruses" – A. R. Taylor, Research Department, Parke, Davis & Co., Detroit, Mich.

"Inactivation of Bacterial Viruses by Physical Methods" – Clarence R. Fuerst, The Princess Margaret Hospital, Toronto, Ont., Canada.

"Ionizing Radiation and its Effects on Animal Viruses" – J. F. McCrea, Yale University, New Haven, Conn.

"Effects of Physical Environment on the Virus of Foot-and-Mouth Disease" – George T. Dimopoullos, Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Greenport, Long Island, N.Y.; Department of Veterinary Science, Louisiana State University, Baton Rouge, La.

Session Chairman: Ernest C. Pollard
Department of Biophysics, Yale University, New Haven, Conn.

2:00 P.M. –
"Correlation of Radiation Effects with Physical and Chemical Changes in Viruses" – Max A. Lauffer, Department of Biophysics, University of Pittsburgh, Pittsburgh, Pa.

"Heat Inactivation of Animal Viruses" – Carl Woese, Biophysics Department, Yale University, New Haven, Conn.

"The Inactivation Function of the Poliovirus-Formalin Mixture" – P.L. Bazeley, Commonwealth Serum Laboratories, Melbourne, Australia.

PANEL DISCUSSION

PROBLEMS TO BE CONSIDERED IN THE INACTIVATION OF VIRUSES

Discussion of effects of inactivation on antigenicity, interference as a factor in masking surviving viruses, test host susceptibility and detection of small amounts of surviving viruses, mutation, implications for preparation of vaccines, problems of isolation of viruses from specimens sent to diagnostic laboratory, and future research needs.

Chairman: Ernest C. Pollard
Department of Biophysics, Yale University, New Haven, Conn.

Discussants: J. W. Beard, Department of Surgery, Duke University School of Medicine, Durham, N. C.
Sven Gard, Karolinska Institutet, Stockholm, Sweden.
H. S. Ginsberg, Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University, Cleveland, Ohio.
W. Henle, Departments of Public Health and Preventive Medicine, School of Medicine, University of Pennsylvania, Philadelphia, Pa.
George K. Hirst, The Public Health Research Institute of the City of New York, New York, N. Y.
Hilary Koprowski, The Wistar Institute, Philadelphia, Pa.
I. W. McLean, Jr., Research Department, Parke, Davis & Co., Detroit, Mich.
J. E. Salk, Virus Research Laboratory, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.
Jesse Chamey, Merck Institute for Therapeutic Research, West Point, Pa.
Fred L. Schaffer, Virus Laboratory, University of California, Berkeley, Calif.

NEW MEMBERS

Elected March 26, 1959

LIFE MEMBERSHIP

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Ryan, V. Gerard, M.D., Clinical Psychiatric Research. Physician-in-Charge, Consultant, Middlesex Memorial Hospital, Middletown, Conn.

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NOTICE TO ACTIVE MEMBERS

In accordance with the Bylaws of the Academy, Article VII, Section 7, the Nominating Committee of the Council is inserting in this April issue a notice to Active Members, quoting Article IV, Section 1 of the Bylaws. Article IV, Section 1 reads as follows:

"**Suggestions by the Members for Nominations.** Suggestions for nominations of Officers of the Academy and Members of the Council, may be sent in writing by any Active Member of the Academy, addressed to the Recording Secretary, with the name of the proposer. To be considered, such suggestions must be received not later than September first of each year."